Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN
PHYTO-
PATHO-
LOGY

V. 4
1964

LIBRARY

CC-0. In Public Domain. Panini Kanya Maha Vidyalaya Collection.

Digitized by Google

Digitized by Arya Samaj Foundation Chennai and eGangotri

गुरुकुल कांगड़ी विश्वविद्यालय, हरिद्वार
पुस्तकालय

विषय संख्या R
पुस्तक संख्या 581.205
आगत पंजिका संख्या N421I
V. 17-19

पुस्तक पर किसी प्रकार का निशान लगाना वर्जित है। कृपया १५ दिन से अधिक समय तक पुस्तक अपने पास न रखें।

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

सन्दर्भ ग्रन्थ
REFRENCE BOOK

यह पुस्तक वितरित न की जाय
NOT TO BE ISSUED

078119

LOGY

UMBER 1

IETY
UTE.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVII 1964 NUMBER 1

6662(B)

078119

PUBLISHED FOR

THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE,
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

In memorium Dr. Rama Rao Sridhar Bada..i—*T. S. Sadasivan* ... 1

Central Himalayan Fungi—I—*S. K. Bose and Emil Muller* ... 3

Studies on four isolates of Rhizoctonia solani Kuehn, the causal agent of black scurf disease of Potato in the Punjab—I —*Madho Singh* 23

On the Physiology of Ascospore formation in powdery mildew of wh at—*H. C. Arya* ... ... [illegible]

... Sclerotium rolfsii to Copper compounds—*D. V. ...thava Reddy and A. Apparao* 35

... records of Plant Diseases from Himachal Pradesh—*H. S. Sohi, S. S. Jain, S. L. Sharma and B. R. Verma* ... 42

Furrow application of fungicides for control of Cotton seedling ...mping-off in Israel—*Z. Solel and G. Minz* ... 46

Fusarium seedling blight of Maize in Rajasthan—*H. C. Arya ...d B. L. Jain* 51

Further studies on foliar necrosis of Potato—*B. Ganguly and S. P. Raychaudhuri* 58

Experiments on Physiologic Specialization in Puccinia penniseti Zimm.—*G. G. Dalela and S. Sinha* 63

Ascomycetes on Celastrus paniculata Willd. II—*S. T. Tilak* ... 66

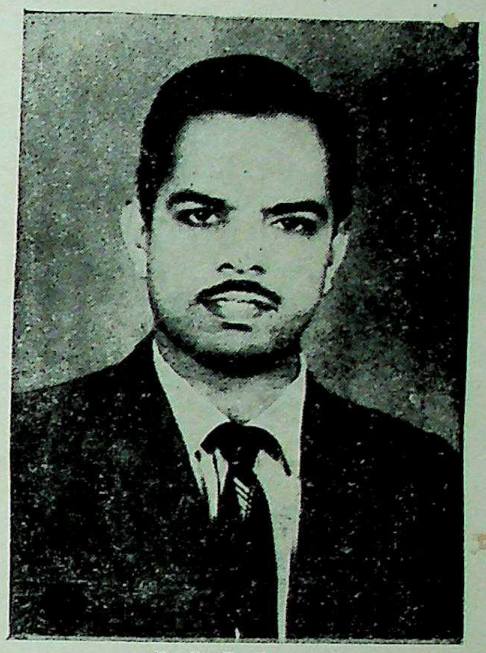
(*Contd. on 3rd cover*)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

1973

IN MEMORIUM
Dr. RAMA RAO SRIDHAR BADAMI



R. S. Badami
1930-1964

The passing away of Dr. Rama Rao Sridhar Badami at a very young age in the prime of his youth and scientific career on the 12th March, 1964, at Bangalore, is a matter of deep regret for most of us who have known him for many years. After graduating from the Mysore University in 1951, he took his M. Sc. from the B. R. College, Agra in 1953. Soon after he came under the powerful influence of the internationally renowned virologist Mr. F. C. Bawden, M. A., F. R. S., Director, Rothamsted Experimental Station at Harpenden in England. Badami took his Doctor of Philosophy degree of the London University working at Rothamsted and much of this work was subsequently published in the *Annals of Applied Biology*. These papers showed in no unmistakable terms Badami's clear thinking and sound contribution in his chosen field of research. Returning to India in 1958 for a short while he was virologist at the Central Potato Research Institute at Simla and soon after came to us at this University and started work as National Institute of Sciences Senior Post-Doctoral Fellow and before long we were able to offer him a position on the staff of this laboratory as Senior Lecturer. This post he held till the last day of his short but useful, creative and intellectual life. In whatever he chose to do whether in Plant Pathology or teaching at the Master's level or helping us in administration he undertook responsibilities with cheerfulness and gusto.

In fact Dr. Badami had initiated a number of investigations in viruses particularly in the field of serology and was guiding the research of two of our students for the Ph. D. degree of this University. These young men are due to submit their theses shortly and reviewing some of their work I was deeply impressed by the large volume of work they have done under the able guidance of Dr. Badami. This is indeed a tribute to Badami's selfless outlook in training young men of science in this country.

I can not recall a single instance when Badami was moody or non-cooperative. Indeed, he had imbibed the true spirit of science and brought to bear thoroughness in whatever he undertook to do. I believe

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

no one likes writing obituaries and more so when one has to write for a dear young colleague. It is doubly depressing. I am voicing the feelings of all my colleagues in the University when I say that we held him in high esteem as a colleague and as a scientist we had learnt to respect him for his integrity, loyalty and intellectual attainments.

Botany Laboratory,
University of Madras. T. S. SADASIVAN

LIST OF PUBLICATIONS

1958 'Changes in the transmissibility by aphids of a strain of cucumber mosaic virus'. *Ann. appl. Biol.*, **46** : 554-562.

1959 'Some effects of changing temperature and of virus inhibition on infection by cucumber mosaic virus'. *Ann. appl. Biol.*, **47** : 78-89.

1959 'Some properties of three virsuses isolated from a diseased plant of Solanum jasminoides Paxt. from India'. (with B. Kassanis,). *Ann. appl. Biol.*, **47** : 90-97.

1959 'The antigenicity of Dolichos Enation Mosaic Virus'. *Curr. Sci.*, **28** : 481-482.

1960 'Sero-diagnostic methods for phytopathological studies'. *J. Indian bot. Soc.*, **39** : 503-530.

1962 'A Survey of cereal and grass virus diseases in India'. (with J.T. Slykhuis,). *J. Indian bot. Soc.*, **41** : 563-570.

1962 'Symposium on Plant and Animal Viruses' (with T.S. Sadasivan). *J. Sci. Industr. Res.*, **21**A : 321-323.

1963 'Some aspects of Plant virus serology', *Bull Nat. Inst. Sci. India* **24** : 166-193.

1963 'Viruses and Parasitism', *J. Indian bot. Soc. Memoir* No. 4.

1664 'A virus disease of cucurbits from India', *Phytopath. Z.* (Accepted for publication).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CENTRAL HIMALAYAN FUNGI—I

S. K. Bose and Emil Müller

(Accepted for publication November 15, 1963)

The present paper is the result of studies conducted by the authors on fungi collected in the central Himalayas. No strict limits have been assigned to the hosts, but the collections, predominantly made in oak forests, comprise of a majority of fungi on the various species of oaks, *viz. Quercus leucotrichophora* A. Camus (=*Quercus incana* Roxb., which is, incidently, antedated by the American species *Q. incana* Bartram), locally known as '*Banj*, *Ban*' or *Ban; Q. glauca* Thunb. subsp. *annulata* Sm., locally known as '*Phariant*, *Phaniat* or *Phaniant*'; and *Q. dilatata* Lindl., locally known as '*Telunj*, *Tilonj* or *Moru*. The collections, including the type specimens of new species, have been deposited in the ETH Herbarium, Zürich.

The authors are greatly indebted to Dr. J. A. von Arx, Phytopathologische Laboratorium, Willie Commelin Scholten, Baarn, Holland, for revising the species.

1. *Ferrarisia quercina* nov. spec.

Ascomata epiphylla, superficialia, late dispersa, mycelio sparso subcuticulo nascentia, 140-190μ latitudine, 18-24μ altitudine. Membrana basalis tenuissima, hyalina, strato unicellulato composita. Membrana tegentis fusca, cellulis plusminusve elongatis, radialiter ordinatis, 2-4μ diam. et circa 2μ altitudine composita. Asci paralleli, dense aggregati, ellipsoidei vel late pyriformi, membrana bitunicata apicaliter crassiore circumdati, 15-22$\times$13-18μ, 8-spori. Ascosporae oblongo-clavatae, circa medium septatae et leniter constrictae, primo hyalinae demum subfuscae, 9-11$\times$4-4.5μ.

Hab. in foliis vivis *Querci glaucae* Thunb. subsp. *annulatae* Sm., Kumaon, Ranikhet (Almora), 28.12.1962, leg. S. K. Bose.

The fly-speck-like, flat, black thyrothecia are scattered on the upper surface of the living leaves and measure 140-190$\times$18-24μ in size. The outer wall of the thyrothecium is composed of brown, radiating cells, 2-4μ in width, equal or longer in length and approximately 2μ in height. Free mycelium is absent round the fruit bodies, but a few hyphae penetrate the cuticle and build under this a sparse hyaline mycelium.

The asci arise from the base of the thyrothecia and are parallel, crowded side by side, obpyriform or egg-shaped, usually flattened at the apex and measure 15-22$\times$13-18μ. Inter-ascicular hyphae are sometimes present as a sparse network towards the base of the tightly packed asci and are more noticeable in the centre of the thyrothecium. The asci

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

are thick-walled at the apex. The thin inner wall extends and projects out when the more rigid outer wall is ruptured by pressure. The ascospores are eight in number, crowded, ellipsoid or oblong-ovate, septate, constricted in the middle with the upper cell somewhat larger in diameter and measure 9-11 × 4-4.5μ. The young ascospores are at first hyaline and full of granular cytoplasm, but the mature ascospores tend to become brownish (fig. 1).

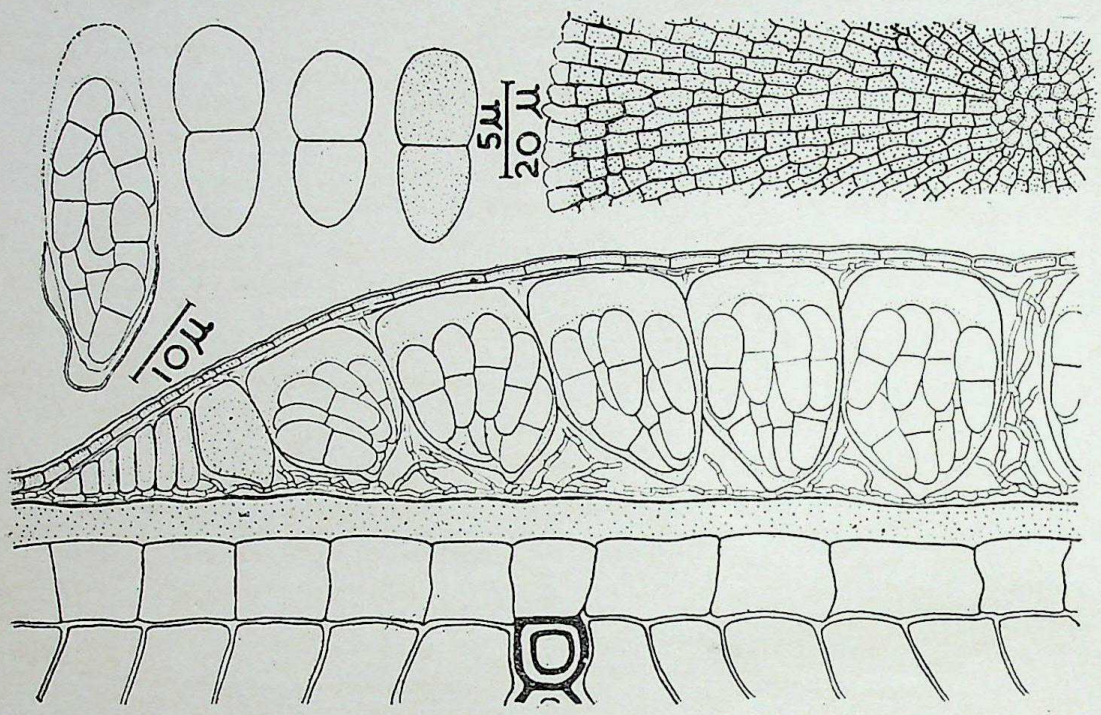


Fig. 1. *Ferrarisia quercina.* Section through a thyrothecium, 1,000×. Upper right — A portion of the thyrothecium viewed from the top, 500×. Upper left — Ruptured ascus, 1,000×. Upper centre — Ascospores, 2,000×.

This ascomycete, at the first glance, appears to be a fly-speck fungus, although it does not belong to the *Schizothyriaceae* as it has diverse characteristics which do not correspond with this family, *viz.*, the radially built thyrothecium is sharply delimited at the periphery; the free hyphae that are lacking on the surface and on the other hand form a thin mycelium within the host; the initially hyaline and later brownish ascospores. The fungus, therefore, fits in within the genus *Ferrarisia* Sacc.

2. *Lembosia decolorans* Syd. *Ann. Mycol.*—**12** : 561 (1914)

Material examined : On living leaves of *Quercus leucotrichophora* collected in Ranikhet, Dist. Almora, U. P., on 16.7.1962; 28.8.1962; 9.10.1962; 20.12.1962; leg. S. K. Bose.

The fungus appears on irregularly round, brown spots on the upper surface of the living leaves and forms sparsely branched, brownish, septate hyphae which are 2-4.5μ in diameter. Round, elliptical or irregular hyphopodia, measuring 4-8μ in diameter, serve to anchor the hyphae and send out simple haustoria within the epidermal cells.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The ascocarps arise from flattened, disk-like, hyphal knots which radiate and elongate to form linear or slightly curved or X-or Y-or + —shaped fruit bodies which are 160-470μ in length and 70-122μ in width. The ascocarp wall is composed of a compact mass of dark tissue which is 6-15μ in thickness and 32-55μ in height. The wall extends on the surface of the leaf in the form of a fringe composed of a single layer of light brown, branched, parallel and radiating hyphae. The basal part of the ascocarp is composed of several layers of thin, light brown hyphae which are 1-2μ in width. A thin layer of similar hyphae is also present across the linear opening at the top of the fruit bodies. This layer disintegrates, along with the paraphysoids, into an indistinct, brownish, slimy tissue upon the maturity of the ascocarps (fig. 2).

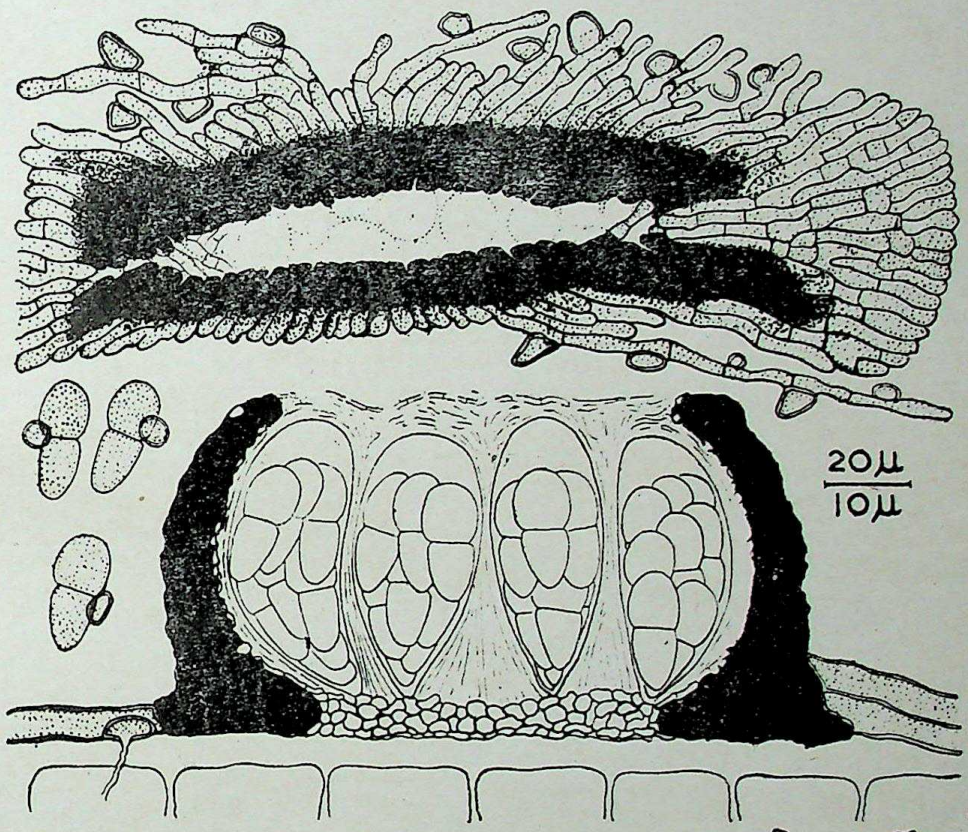


Fig. 2. *Lembosia decolorans*. Section through an ascocarp, 1,000×. Left—Germinating ascospores with hyphopodia, 1,000×. Above—Ascocarps viewed from the top, 500×.

The asci are sessile, obpyriform, obovate or cylindrical-egg-shaped, broadly rounded and thickened at the apex, and measure 35-47×11-18μ in size. The ascospores are eight in number, overlapping three-seriate or crowded, ellipsoid, bicelled, constricted at the septa and 11-18×3-7μ in size. The ascospores are hyaline in early stages but ultimately become brown. These germinate by giving out a hyphopodium close to the septum. The paraphysoids are filiform, hyaline and mingle with the light brown hyphal covering at the top but ultimately become indistinct and gelatinous due to subsequent compression by the enlarging

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The fungus closely approximates *Lembosia decolorans* Syd., which has been recorded from the Philippines on the leaves of *Quercus ovalis* Blanco [=*Lithocarpus ovalis* (Blanco) Rehder] and is considered to be identical.

3. *Johansonia arxii* nov. spec.

Mycelium superficiale hypophyllum, indistinctum, hyphis ramosis, septatis, subhyalinis vel hyalinis, ca 2μ crassis compositum. Ascomata superficialia, dispersa, supra subiculum nascentia, setis erectis simplicibus, septatis, atro-brunneis, ad 150μ longitudine, et 4-5μ crassitudine circumdata, disciformia, orbiculata, brunnea, 300-400μ diam. et 60-70μ altitudine. Excipulum 5-10μ crassitudine, subfuscum cellulis elongatis formatum. Integumentum cellulis applanatis, fuscis, non coharentibus compositum. Asci numerosi, dense aggregati, paralleli dispositi, late clavati, crasse bitunicati, 8-spori, 30-37×10-13μ, paraphysoidibus filiformibus, apicaliter clavatibus circumdati. Ascosporae clavatae, circa medium septatae, constrictae, hyalinae, 7-10×2-3μ.

Hab. in foliis vivis *Querci dilatatae* Lindl., Kumaon, Pithoragarh, 31-12-61, leg. M.C. Pande.

The fungus grows superficially on lower surface of the leaves and builds a thin, diffused, widely extending mycelium which is composed of subhyaline to hyaline hyphae, measuring approximately 2μ in diameter. The mycelium thickens layer by layer into a 10-15μ high subiculum composed of light brown hyphae. On this a disk-like thyrothecium is formed. The latter measures 300-400μ in diameter and 60-70μ in height. The thyrothecium is surrounded by a few dark, more or less pointed bristles which arise out of the subiculum and are upto 150μ in length and 4-5μ in diameter. The basal layer of

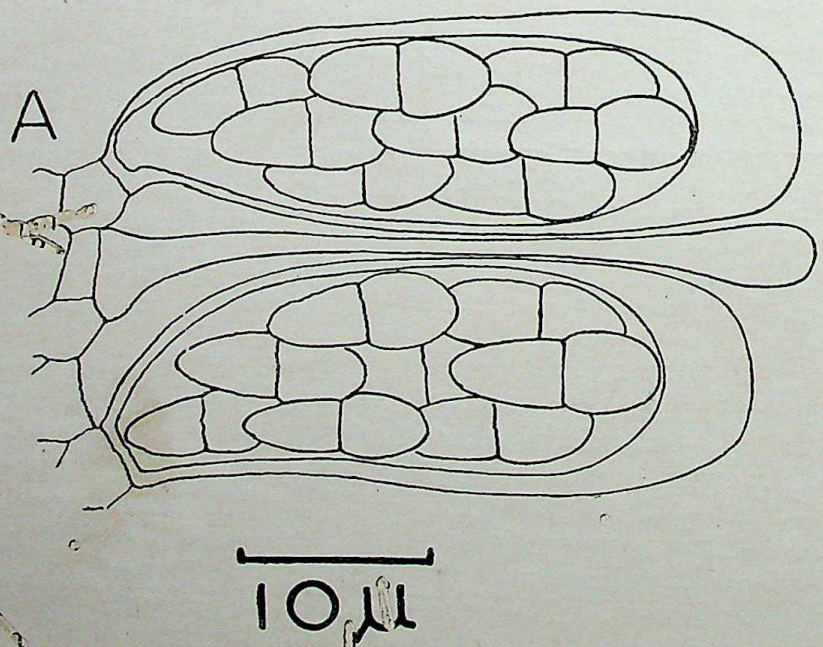


Fig. 3.A— *Johansonia arxii*, Asci, 2,000×.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the hypothecium is 4-6μ in thickness and is composed of short, light brown, thickly interwoven hyphal filaments. At the periphery, the fruit body is edged by a wall of small, identically light brown, thin-walled cells. At the top the fruit body is covered by a partly torn and partly dissolving roof of brown plate-like cells (Fig. 3).

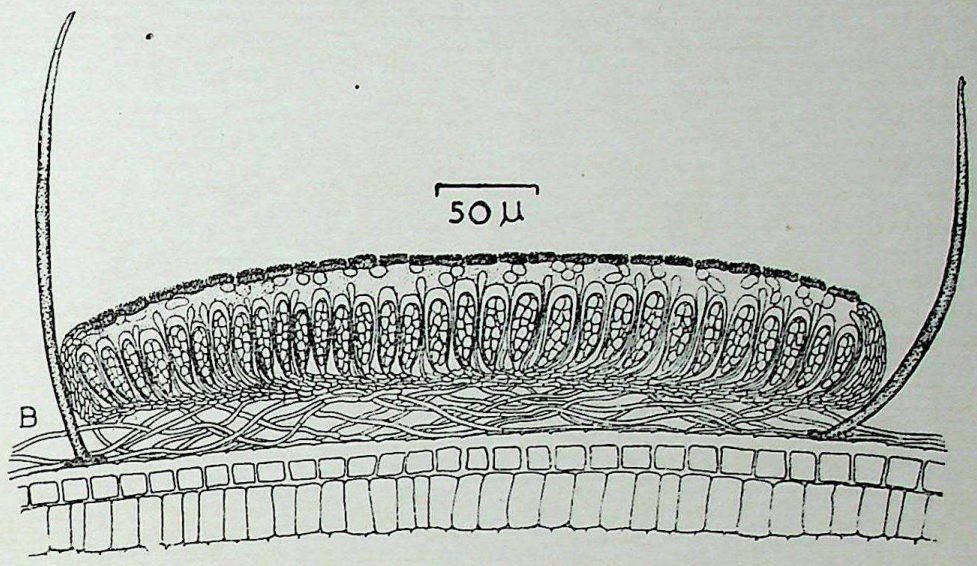


Fig. 3 B—Section through an ascocarp, 250×.

The asci are thickly parallel, club-shaped, variable in length, mostly with short stipes, bitunicate, distinctly thick-walled at the apex, 8-spored and measure 30-37×10-13μ in size. The asci stand in a hyaline hypothecium, initially composed of hyphal cells, these later on dissolve into a slime, and are surrounded by thread-like apically club-shaped paraphysoids which subsequenly also become slimy. The ascospores are biseriate, hyaline, oblong-ellipsoid, septate, approximately in the middle and measure 7-10×2-3μ. The upper cell is rounded at the apex and is wider than the lower cell.

This fungus has been named after Dr. J. A. von Arx for his great help to the authors.

4. *Sphaerulina quercifolia* nov. spec.

Perithecia in maculis rotundatis vel irregulariter circumdatis, fuscis nascentia, immersa, globosa vel ellipsoidea, ad 180μ diam. Paries peritheciorum 8—35μ crassitudine, apicaliter crassior et applanata, ostiolo rotundato aperta. Asci cylindracei vel cylindraceo-clavati, 50—110×16—18μ, 8-spori. Paraphysoides nullae. Ascosporae cylindraceae, parallelae, rectae vel leniter curvatae, 5-10-septatae, hyalinae, 50—70×4—4.5μ magnitudine.

Hab. in foliis vivis *Querci leucotrichophorae* A. Camus (=*Querci incanae* Roxb.) Kumaon, Ranikhet (Almora) 18.7.1962. leg. S. K. Bose.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

On the upper surface of the living leaves, the fungus causes circular, angular or irregular, light brown spots with brown margins. The ascocarps are scattered, immersed, round or oval, upto 180μ in diameter and open on either the upper or the lower surface of the leaves. The ascocarp wall is composed of elongated, brown, thick-walled polyhedral cells which are upto 16μ in length. The wall is 8 – 35μ in thickness towards the sides and base and usually somewhat thicker and flattened at the apex. The ostiole is simple and schizogonic (fig. 4).

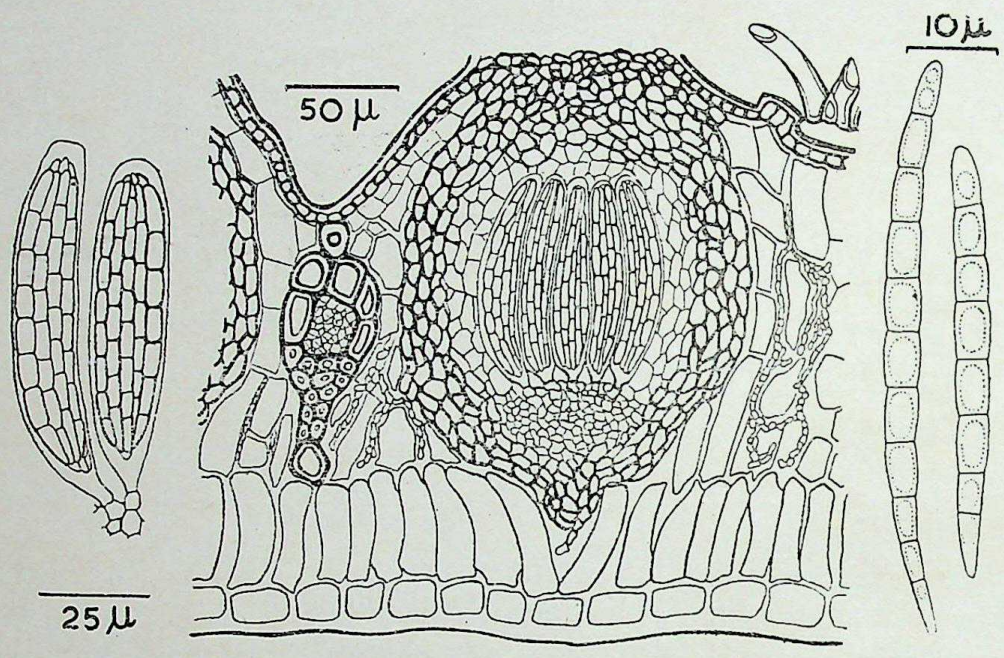


Fig. 4. *Sphaerulina quercifolia*. Section through an ascocarp, 125×. Left—Asci, 250×. Right—Ascospores, 500×.

The asci are aparaphysate, cylindrical or cylindrical-clavate, rounded or flattened at the apex and arise from a cushion-like subhymenial tissue composed of hyaline pseudoparenchymatous cells which are upto 10μ in diameter. The space between the brown ascocarp wall and the asci is occupied by several layers of elongated, pseudoparenchymatous tissue. The asci are usually 50—90μ, sometimes upto 110μ in length and 16—18μ in diameter. The ascospores are eight in number, parallel, cylindrical, straight or slightly curved, 5—10 septate, rounded at the apex and slightly tapering below. The spores are lightly constricted at the septa and measure 50—70×4—4.5μ in size.

5. *Coccoidella quercifolia* nov. spec.

Stromata amphigena, sine maculis, irregulariter dispersa, pleumque hypophylla, rotundata vel elongata, 140—250μ latitudine et 60—75μ altitudine, centro hypostromatice innata. Loculi dense stipati, globosivel piriformi, 70—90μ diam. apicaliter poro rotundato pertusi, peritibus 8—12μ crassis, fuscis circumdati. Asci cylindracei, in vertive late rotundati, 8-spori, 45—55×10—12μ. Paraphysoidibus filiformibus circumdati. Sporae distichae, oblongae vel oblonge-clavatae, circa medium septatae, [illegible]fuscae, 14—16×4—4.5μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

[illegible] in foliis vivis *Querci glaucae* Thunb. subsp. *annulatae* Sm., Kumaon, Ranikhet (Almora) near Windy Haugh, 28.12.1962, leg. S. K. Bose.

The thinly scattered superficial stromata are formed on the lower surface of the living leaves without causing any spots. These are flat disc-like, circular in outline or somewhat elongated, measuring 140—250μ in diameter and 60—75μ in height. Beneath their centrum, these are anchored to the leaf tissues by means of a foot-like hypostroma which penetrates the dissolved epidermis by means of a plectenchymatic tissue, killing the host cells in a wide circle and also penetrating into numerous mesophyll cells. In the region of the epidermis, the hypostroma is composed of elongated, somewhat thin-walled, light brown cells which gradually diffuse within the tissues of the leaf into a light coloured delicate mycelium.

The fruit body-like loculi are spherical or pear-shaped and measure 70—90μ in diameter. The wall is 8—12μ in thickness, composed of somewhat thick-walled brown cells, measuring 4—6μ in diameter, and open at the top by means of a round pore. The cells of the wall are usually round in the upper part, becoming elongated in the lower region.

The asci are 8-spored, cylindrical, broadly rounded at the apex and measure 45—55 × 10—12μ. The ascospores are biseriate, elongated ellipsoid, slightly constricted, septate approximately in the middle, brownish or greyish-yellow and 14—16 × 4—5μ in size. The asci are surrounded by dense, thread-like paraphysoids (fig. 5).

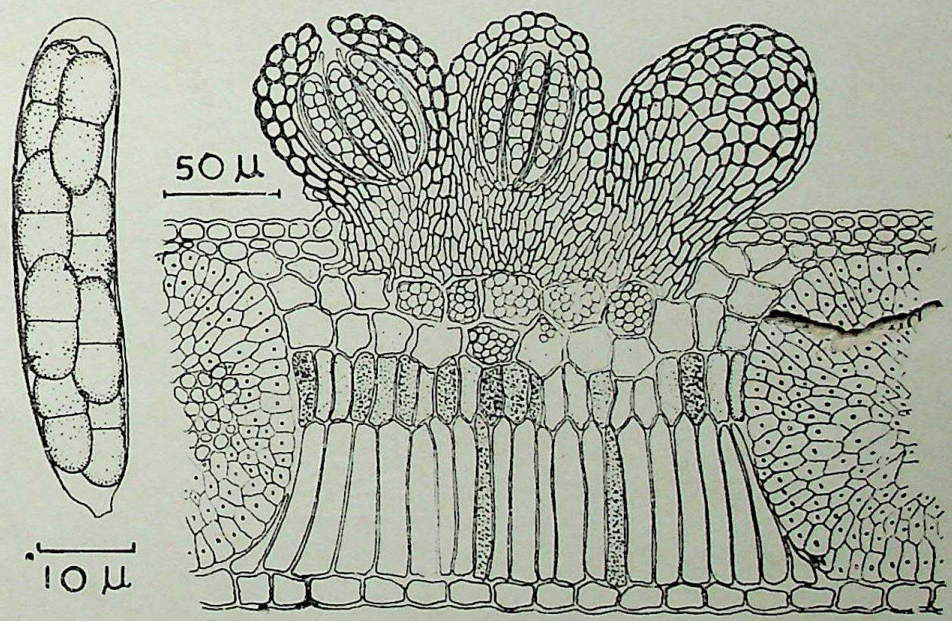


Fig. 5. *Coccoidella quercifolia*. Section through a stroma, 250× and ascus, 1,000×.

According to Müller and v. Arx (1962), the genus *Coccoidella* v. Höhn. belongs to the *Venturiaceae* and is closely related to *Coccoidea* P.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Henn. whose type species, *Coccoidea quercicola* P. Henn. likewise parasitizes the leaves of *Fagacea*, among these also those of *Quercus glauca* Thunb. occurring in Japan. *Coccoidea quercicola* differ from *Coccoidella quercifolia* especially in the size and septation of ascospores which measure 11-15 × 5-7μ and are septate near the lower end.

6. *Dimerium venturioides* (Sacc. et Berl.) Thesis.—Beih. Bot. Zbl., Abt. 2, **29** : 49 (1912).

MATERIAL EXAMINED : On living mycelium of *Meliola melanochaeta* Syd., growing on leaves of *Quercus leucotrichophora* A. Camus, collected in Paperseli (Epworth Bungalow), Almora, U.P. 11.11.1962, leg. A. J. Roy.

The mycelium ramifies over the hyphae of *M. melanochaeta* and is composed of subhyaline or light yellow, septate hyphae, measuring 2-4μ in diameter. The ascocarps are superficial, attached to the host mycelium by means of a dense mass of hyphae and are yellowish-brown or brown, globose, papillate at the apex, measuring 60-80 × 50-70μ in size. The ascocarp wall is composed of a single layer of polyhedral cells which are 4-8μ in diameter. The papillate apex is 15-22μ in width and is composed of elongated cells (fig. 6).

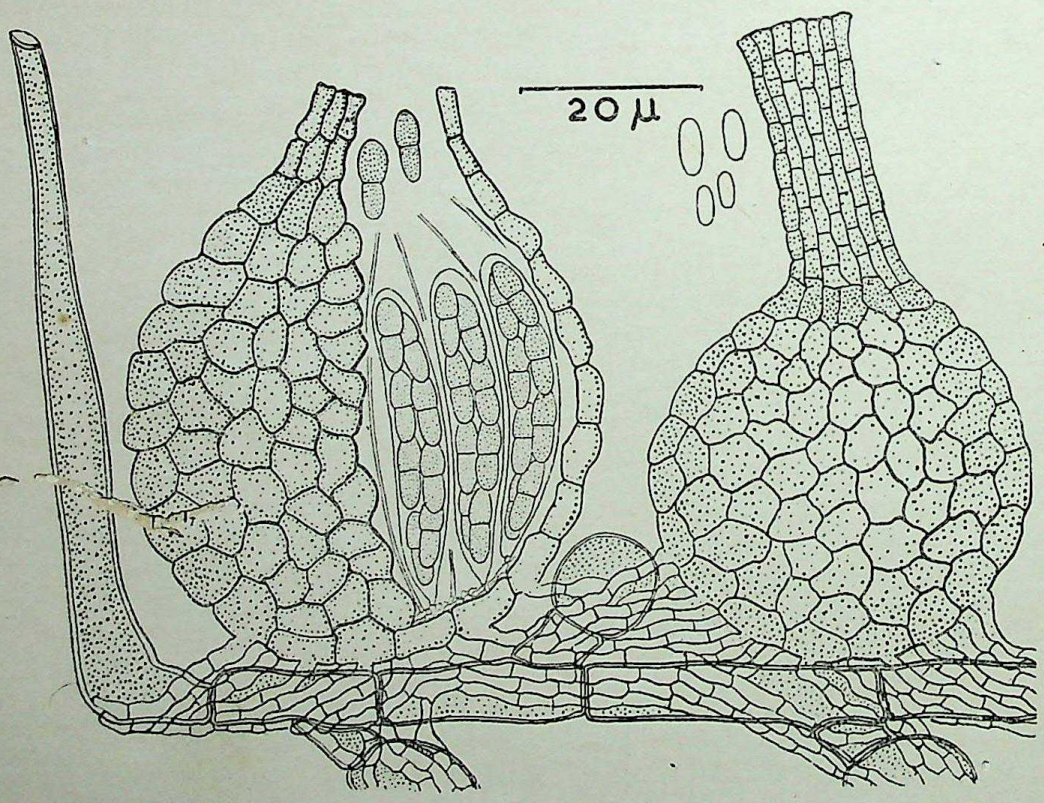


Fig. 6. *Dimerium venturioides*. Ascocarp, pycnidium and pycnidiospores, 1,000×.

The asci are cylindrical, subsessile with a double membrane which is rounded and thickened at the apex, and measure 25-35 × 5-7μ. The ascospores are eight in number, biseriate, oblong-ellipsoid, brown, bicelled and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

constricted at the septum with the upper cell slightly broader than the lower and are $10-15 \times 5-7\mu$ in size. The paraphysoids are very thin, sparse and become evanescent in the mature ascocarps.

Pycnidia similar to the ascocarps, but smaller, lighter in colour and often with a long neck, appear on the same mycelium. These are $30-50\mu$ in diameter and are composed of polyhedral cells which elongate in parallel rows in the neck where they become upto 33μ in length and $10-15\mu$ in diameter. The pycnidiospores are pale yellow, unicellular, ellipsoid and $4-6 \times 2-2.5\mu$ in size.

The fungus agrees closely with *Dimerium venturioides* which occurs in the tropics. Hansford (1946) has discussed this fungus along with the synonyms. Müller (1954) has described a morphologically similar fungus from Europe under the name *Dimerium maculosum* (Speg.) Müller.

7. *Wentiomyces javanicus* Koorders—Verh. K. Ak. Wet. Amsterdam II, 13 (4) : 168 (1907).

MATERIAL EXAMINED : Empty perithecia of *Neophacidium quercus* (Chona, Munjal et Kapoor) Müller et Ahmed, on fallen leaves of *Quercus leucotrichophora* A. Camus, collected in Government Gardens, Chaubattia (Ranikhet), Almora, U.P., on 15.10.1962, leg. T. C. Sharma.

The fungus usually appears within and sometimes on the surface of empty fruit bodies of *Neophacidium quercus*. The ascocarps are globular, somewhat bluntly papillate at the apex and measure $45-55\mu$ in diameter. The membraneous ascocarp wall is composed of polyhedral, light brown cells, measuring $6-12\mu$ in diameter, and are beset with simple, flexuous, subhyaline or light brown appendages which arise from the sides. The appendages are upto $60\ \mu$ in length and $6-12\mu$ in diameter. The apical portion of the perithecium is composed of smaller cells, measuring $2-4.5\mu$ in diameter. A ring of 4–6 erect, brown appendages are present in this region. These erect appendages are septate, $14-18 \times 3.5-4.5\mu$ in size and are branched and expanded at the apex. The ascocarps are anchored to the substratum by means of a number of septate, light brown hyphae, arising from the base.

The asci are aparaphysate, cylindrical or cylindrical-clavate, truncate or subsessile at the base, rounded at the apex and measure $25-29 \times 5-6.5\mu$. The ascospores are eight in number, bi-seriate, hyaline, oblong-ellipsoid, bicelled, measuring $5.5-6.5\mu$ in length and $2-2.5\mu$ in diameter with the lower cell slightly narrower in width (fig. 7).

Although this fungus is somewhat smaller in every respect than given in the description of *Wentiomyces javanicus* it agrees, so closely, with all the salient features of this species that it can hardly be considered to be a different species.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

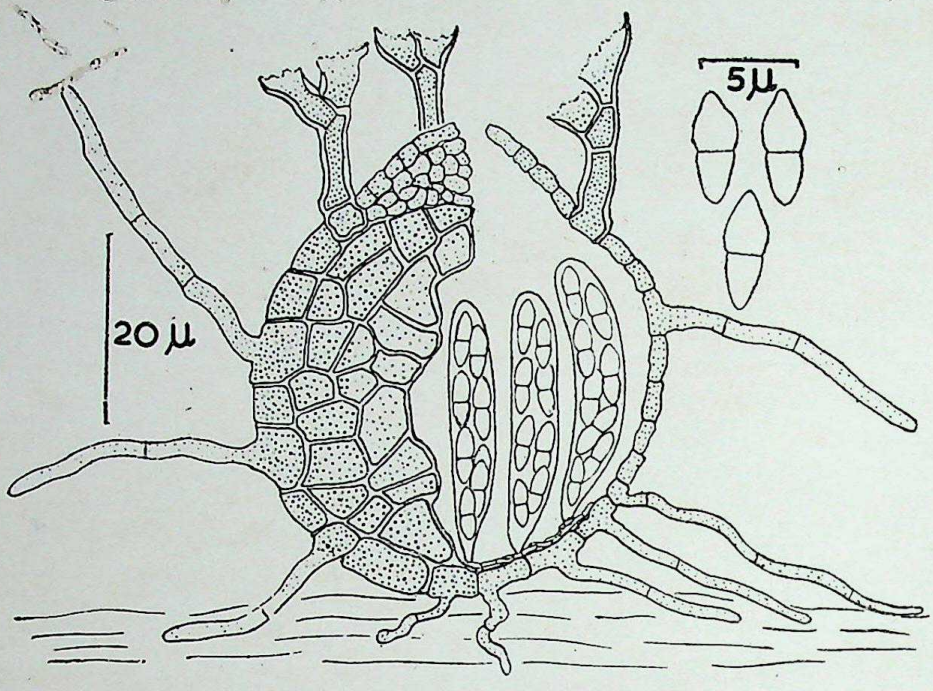


Fig. 7. *Wentiomyces javanicus*. Ascocarp, 1,000× and ascospores, 2,000×.

8. *Coccochora kusanoi* (P. Henn.) v. Höhn.—Sitzungsber. K. Ak. Wiss. Wien, math.-nat. Kl., 1. Abt., **118** : 53 (1909) (Fragment no. 444).

MATERIAL EXAMINED : Living leaves of *Quercus glauca* Thunb. subsp. *annulata* Sm., collected in Chaubattia (Ranikhet), Almora, U.P., on 10.9.1962, leg. A. J. Roy.

The fungus appears during the monsoon and ramifies beneath the cuticle of the upper surface of living leaves, forming reddish-brown spots which are upto 4 mm. in diameter. The subcuticular, hyaline to reddish-brown hyphae measure 0.5 – 2μ in diameter. Later on, a number of ascocarps appear on each spot. During the formation of the ascocarps, brown hyphae, approximately 0.5μ in diameter, ramify intercellularly between the epidermal cells. The latter elongate and proliferate into irregular, polyhedral cells. These bulge beneath the cuticle as black, sub-hemispherical structures and later on, at the side of each, a projecting U-shaped neck is formed laterally. The mature ascocarps are upto 450μ in diameter and 75 – 100μ in height. The ascocarp wall is 12—20μ in thickness and is composed of isodiametric or elongated, dark brown, thick-walled cells, approximately 5μ in diameter, interspersed with the remains of the epidermal cells. The base of the ascocarp is composed of the lower part of the degenerated epidermal cells in which the light brown, thin-walled, polyhedral cells of the fungus grow. Towards the edge, the ascocarp wall is composed of elongated polyhedral cells. The U-shaped neck is 100 – 150μ wide at the apex, 80 – 110μ in height and is full of proliferated, polyhedral epidermal cells which ultimately degenerate to form the pore.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The horizontal, aparaphysate, bitunicate asci arise from the edge of the ascocarp cavity, opposite the pore and are cylindrical or cylindrical-clavate, measuring 135—185 × 35.5—46.5 μ. The wall, when crushed in water, disintegrates into a slimy matter. The ascospores are eight in number, biseriate, thick-walled, ellipsoid, bicelled, slightly constricted and septate in the lower part, hyaline at first, slowly turning brown and measure 35.5—49 × 15.5—22 μ (fig. 8).

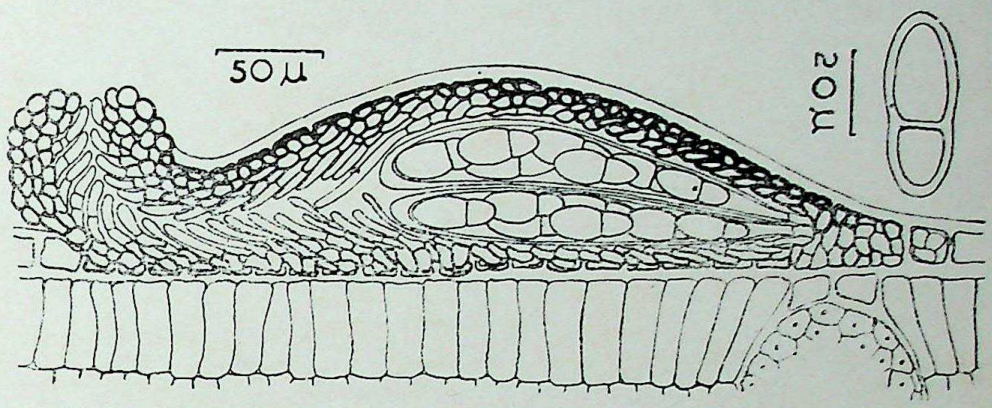


Fig. 8. *Coccochora kusanoi*. Section through an ascocarp, 250 ×. Right—Ascospore, 500 ×.

There is no doubt that this peculiarly built psendosphaerial fungus is identical with *Coccochora kusanoi*. This ascomycete has been described by Hennings (1904) as *Dothidella kusanoi* from Japan where it grows as a leaf parasite of the typical *Quercus glauca*. Its fruit body grows epidermally and not, as Theissen and Sydow (1915) have described, subcuticularly. Moreover, the facts that the peculiar ostiole is formed at the side, and that the axis of the fruit body lies horizontally, have been observed neither by von Höhnel (1910) nor by Theissen and Sydow (1915). The ascospores remain hyaline for a long time and in the above material one can observe only a few ascospores which have turned brown, whereby, as rightly described by Theissen and Sydow, both the spore cells become identically coloured.

9. *Microthyrium biciliatum* nov. spec.

Mycelium subcuticulam nascentem vel inter cellulas epidermicas penetrantem, hyphis subfuscis, reticulate ramosis compositum. Ascomata epiphylla, superficialia, dispersa vel bina vel complura approximata vel subaggregata, rotundato, 160—240 μ latitudine, 35—45 μ altitudine; centro poro rotundato, 10—20 μ late aperta. Membrana basalis tenuissima, hyphis hyalinis composita. Stratum tegente cellulis applanatis, rectangulatis, ca 6 μ diam., fuscis, radialiter ordinatis compositum. Centrum ascomatae corpore conico sterile ex paraphysoidibus compositum. Asci cylindracei, 44—55 × 5—7 μ, 8-spori, paraphysoidibus filiformibus circumdati. Sporae fusoideae, infra medium septatae, hyalinae, 11.5—13.5 × 2—2.5 μ, apicaliter setulis duibus, 9—11 μ longis ornatae.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Hab. in foliis emortuis *Querci leucotrichophorae* A. Camus, Kumaon, Chaubattia (Ranikhet), Almora, 16.11.1962, leg. A.J. Roy.

The shield-shaped thyrothecia are scattered on the upper surface of the dead leaves and measure 160–240×35–45 μ. The wall is composed of a layer of thick-walled, radiating, flattened, polyhedral or rectangular cells, measuring upto 6 μ in length. The apex is slightly raised and is composed of a loose layer of thin-walled cells which ultimately disintegrate to form an ostiole, measuring 10–20 μ in width. The base of the thyrothecium is composed of several layers of subhyaline or light brown hyphae, measuring approximately 2 μ in diameter which ramify within the cuticle and so metimes penetrate into the interspaces of the epidermal cells.

The asci are cylindrical, tapering at the apex and base, with a double membrane, densely crowded, converging towards the ostiole and measure 44–55×5–7 μ. The paraphyses are filiform, septate, hyaline and approximately 1 μ in diameter. The central portion of the centrum is filled with a triangular column of paraphysoidal threads. The ascospores are eight in number, biseriate, hyaline, spindle-shaped, bicelled and measure 11.5–13.5×2–2.5 μ. The upper cell is somewhat longer than the lower and is beset with a pair of thin, hyaline cilia which are 9–11 μ in length (fig. 9).

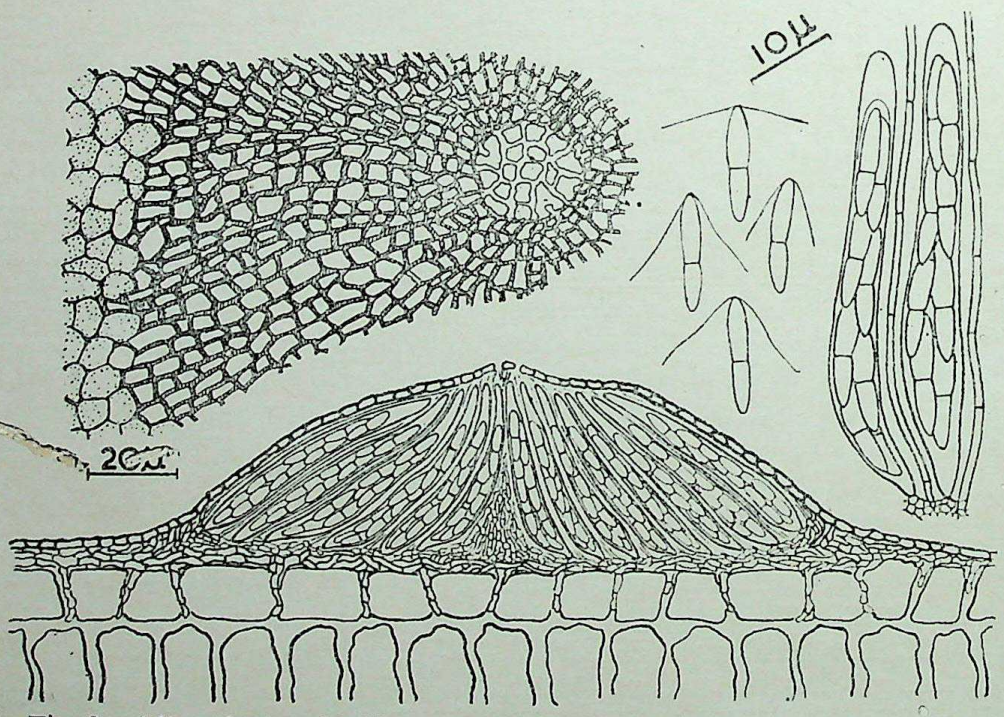


Fig. 9. *Microthyrium biciliatum*. Section through a thyrothecium 500×. Upper left—A portion of the thyrothecium viewed from the top, 500×. Centre—Ascospores, 1,000 ×. Right—Asci, 1,000×.

This ascomycete, without doubt, belongs to the family *Microthyriaceae*. According to the key given by Müller and von Arx (1962), two genera come into consideration—*Microthyrium* Desm. and *Cyclotheca* Theiss. The latter encompasses parasitic species whose mycelium

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

is discernible in the cuticle, often penetrating still deeper in the host tissues, whereas the typical species of *Microthyrium*, so far known, occur only on dead parts of the host and either do not penetrate the tissues at all or do so to a very limited extent. The fungus described above, takes an intermediate position in that it colonizes the host tissues somewhat more intensively, but on the other hand, it grows saprophytically and the morphology of its thyrothecium reminds one about the other species of *Microthyrium* that also grows on the leaves of various species of *Quercus*. Easily next in relation comes *Microthyrium ilicinum* de Not. with somewhat smaller fruit body, absolutely superficial mycelium and with ascospores carrying two pairs of apical cilia.

10. *Microthyrium pieridis* nov. spec.

Mycelium indistinctum, superficialium, ex hyphis subhyalinis compositum. Ascomata, superficilia, epiphyllia, dispersa, scutiformia, rotundata, 150–200 μ latitudine et circa 40 μ altitudine, centro pororotundato, 10–15 μ lato aperta. Membrana basali tenuissima, hyalina: stratum tegente cellulis elongatis, 8–12×1.5–2.5 μ fuscis, radialiter ordinatis compositum. Centrum ascomatae corpus conico ex paraphysoidibus nascentium. Asci cylindracei-saccati, 38–42×6–8 μ, 8-spori. Sporae distichae, fusoideo-clavatae, infra medium septatae, hyalinae, 11–13×2.5–3 μ, apicaliter setulis duibus ornatae.

Hab. in foliis emortuis *Pieridis ovalifoliae* D. Don., Kumaon, Bindsar (Almora), 22.5.1957, leg. E. Müller, B.D. Sanwal.

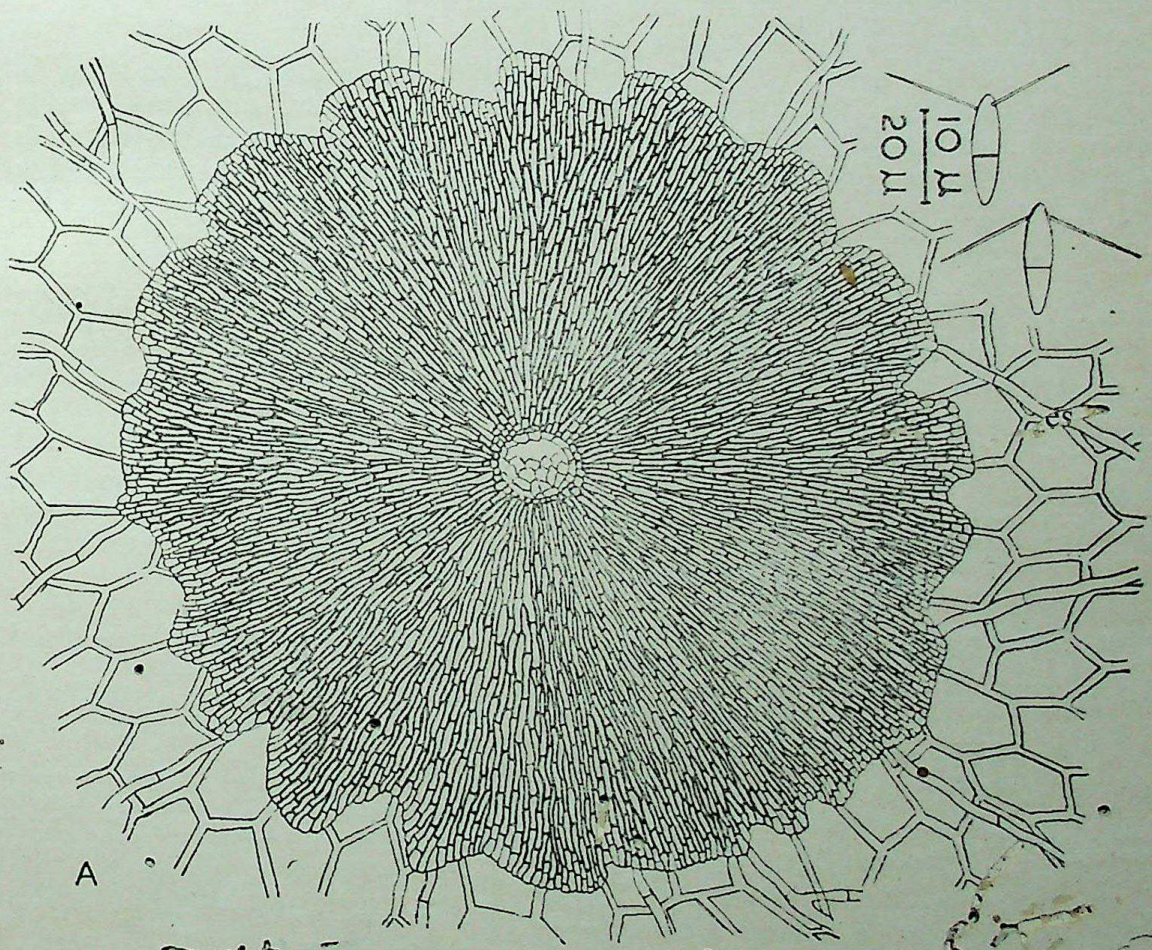


Fig. 10a. *Microthyrium pieridis*. A.—Surface view of a thyrothecium, 500×. Right—Two ascospores, 1,000×.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

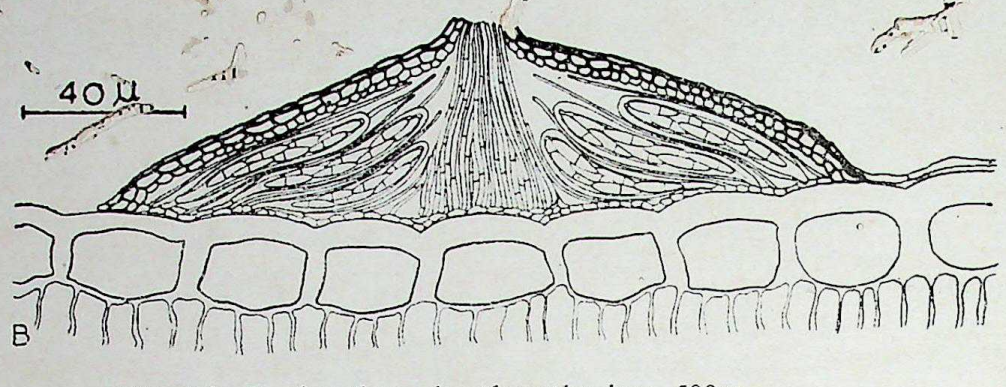


Fig. 10b. Section through a thyrothecium, 500×.

The mycelium is barely discernible and diffused, and on this the shield-like thyrothecia are formed. These are circular at the margin, measuring 150–200 μ in diameter and are approximately 40 μ high at the centre. These adhere to the cuticle by means of a delicate basal wall which is usually composed of a layer of flat thin-walled, hyaline cells. At the apex, the thyrothecium opens by means of a pore, measuring 10–15 μein width. The sharply radial upper wall is composed of 2–3 layers of plate-like, somewhat thick-walled cells. Viewed from above, these cells appear to be elongated and measure 8–12×1.5–2.5μ. The serile centrum, composed of a blunt cone of adhering paraphysoids, is surrounded by the asci in a ring-like manner. The asci obliquely incline towards the pore and are cylindrical, usually sac-like in the lower part, measuring 38–42×6–8 μ in size.

The ascospores are eight in number, biseriate, fusiform-spindle-shaped, septate somewhat below the middle and are beset with two cilia, measuring 10–15 μ in length. The cilia are attached towards the sides, immediately below the upper end. The ascospores are hyaline and 11–13×2.5–3 μ in size. The asci are surrounded by thread-like paraphysoids which ultimately become slimy (Fig. 10).

Microthyrium pieridis differs from *Microthyrium biciliata* and from other species like *M. microscopicum* Desm., *M. ilicina* de Not., and *M. macrospora* (Sacc.) v. Höhn., foremost in the structure of the upper wall. In all these cases, the upper layer is, of course, sharply radial, but viewed from above, the cells are typically more or less quadratical, whereas in *Microthyrium pieridis*, the cells are, however, remarkably elongated. As in *Microthyrium biciliata*, the ascospores carry two stiff cilia, these are not attached at the apex but are stiff and inserted somewhat laterally.

11. *Micropeltis congonensis* Beeli—Bull. Jard. bot. Brux. **8**: 4 (1923).

Material examined: On living leaves of *Euonymous pendulus* Wall. collected in Ranikhet (Dhobi Ghat), Almora, U.P., on 10.8.1962, leg. M.C. Pande.

The subhemispherical thyrothecia are scattered on the lower surface of the living leaves and measure 500–650×140–170 μ. The

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

thyrothecial wall is composed of three layers. On the outer surface, there is a single layer of brown hyphae, covering three or four layers of hyaline, septate hyphae, forming the middle region. The lowermost part consists of two layers of yellowish-brown hyphae. All these hyphae are approximately 2 μ in diameter. The flattened base of the thyrothecium is composed of hyaline hyphae. Towards the edges, the wall of the thyrothecium diffuses into a short net-work of hyaline hyphae which anastomose obliquely or at right angles. The thyrothecia are without any ostiole in the beginning, but ultimately open at the top by a schizogonous pore, measuring 40—50 μ in width (fig. 11).

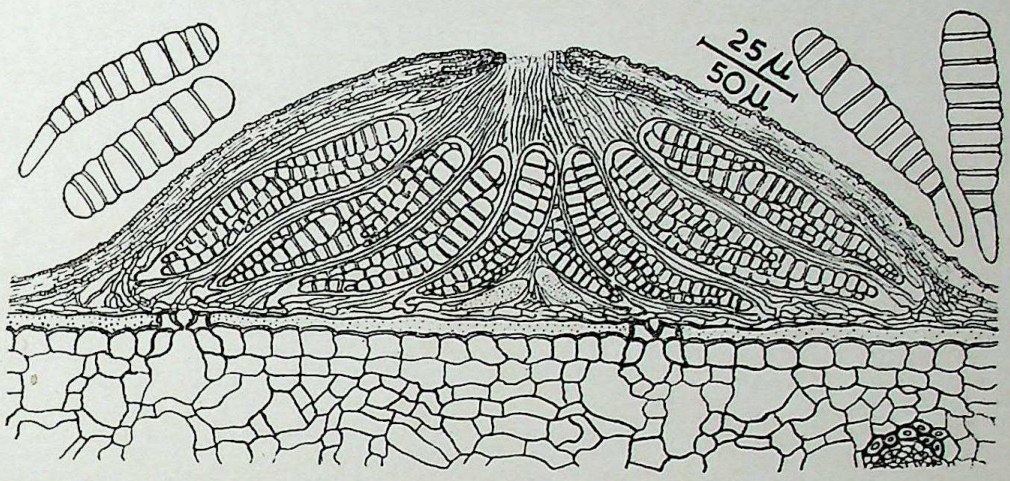


Fig. 11. *Micropeltis congonensis.* Section through a thyrothecium, 250×. Above—Ascospores, 500×.

The asci are paraphysate, bitunicate, cylindrical or cylindrical-clavate, thick-walled and rounded at the apex, crowded, converging towards the ostiole and 110—170×17—26 μ in size. The ascospores are overlapping three-seriate or crowded, hyaline, cylindrical-club-shaped, constricted at septa, broadly rounded at the apex and tapering below. The septa are 8—13 in number but may be fewer in the smaller ascospores. The mature ascospores are 25—62×8.5—13 μ in size. The paraphysoids are hyaline, filiform, septate, approximately 1.5 μ in diameter and are usually scanty in mature ascocarps due to compression by the thickly crowded asci.

The fungus described above approximates *Micropeltis congonensis* and is considered to be identical.

12. *Actinopeltis ciliaris* nov. spec.

In mycelio *Lembosiae decolorantis* parasitans; mycelium late expansum sed etiam sub lente valida non vel vix perspicuum ex hyphis ramosis, suberectis, subhyalinis compositum. Ascomata dispersa, rotundata, 200—350 μ diam. et 22—30 μ altitudine. Membrana basalis tenuis subfusca, cellulis applanatis, prismaticis, 3—4 μ latitudine, 4—5 μ longitudine et 2 μ altitudine, radialiter ordinatis composita. Stratum tegente simile, cellulis fuscis compositum, apicaliter poro rotundato 8—12 μ diam. setulis pluribus rectis, fuscis, 12—16×2—3 μ orna-

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

apertum. In paritibus exterioribus ascomatorum membrana basalis et membrana tegentis strato unicellularo coniugata. In partibus centralibus ascomatorum asci cylindracei-clavati, 36–40×6.5–8 μ 8-spori nascentes. Sporae cylindraceae, supra medium septatae, hyalinae, 12–15×2–2.5 μ, apicaliter 6-ciliatae. Paraphysoides fibrosae.

Hab. in foliis emortuis *Querci glaucae* Thunb. subsp. *annulatae* Sm., Kumaon, Ranikhet (Almora) 30.12.1962, leg. S.K. Bose.

The fungus appears on both surfaces of dead leaves of *Quercus glauca* subsp. *annulata* as a parasite on the hyphae of *Lembosia decolorans* forming flat, disc-like structures composed of brownish cells which ultimately develop into mature lens-shaped thyrothecia, measuring 200–250 μ in diameter and 22–30 μ in height. The base of the thyrothecium is composed of a single layer of light brown cells, radiating from a central, concentric, ring-like structure which had been originally formed round the host cells. The cells of the basal layer are upto 4 μ in width, equal or usually longer in length and approximately 2 μ in height. The upper wall of the thyrothecium is composed of a layer similar to the basal layer, also radiating from a central concentric ring, but the cells are darker. The central concentric ring, in this case, is more complex than its counterpart in the basal layer and is composed of 1–3 rings of dark thick-walled cells and are beset with brown, unseptate, stiff, pointed bristles, measuring 12–16×2–3 μ in size. The innermost bristles are lighter in colour and conically converge above the ostiole. The setae are sometimes not discernible, especially in early stages. The ostiole is usually 8–12 μ in diameter. The outer and the basal layers of the thyrothecia are joined at the edges. The

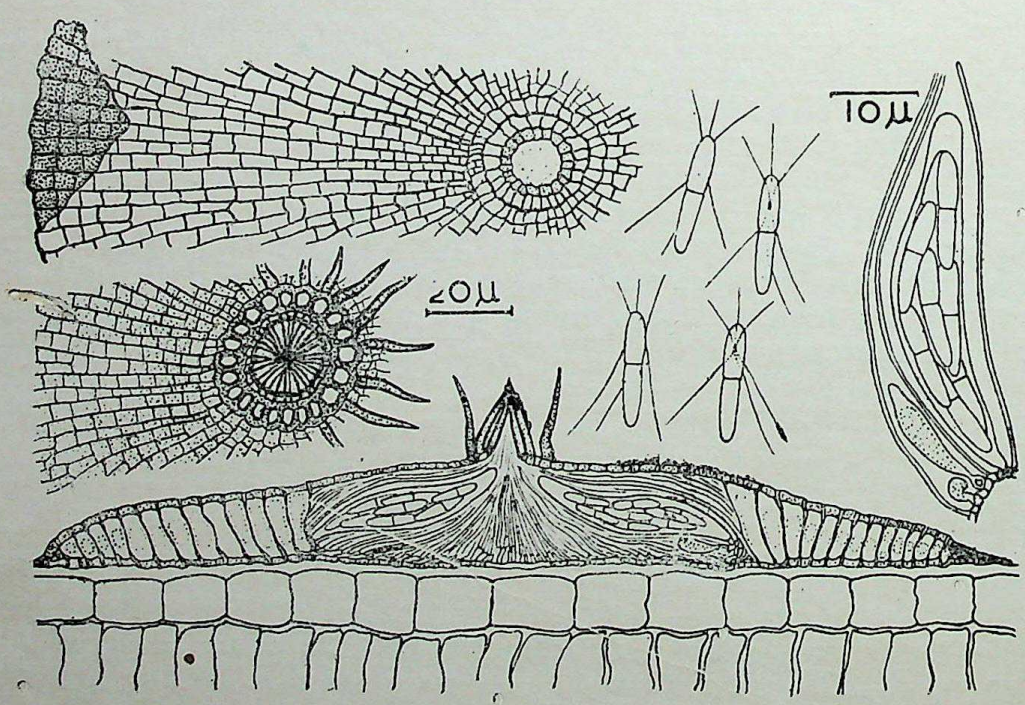


Fig. 12. *Actinopeltis ciliaris*. Section through a thyrothecium, 500×. Upper left—A portion of the basal layer of the thyrothecium, 500×. Left middle—A portion of the upper wall of the thyrothecium, 500×. Upper right—asci and ascospores, 1,000×.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

upper layer is raised up by closely packed, single-celled, hyaline, thick-walled, refractive, upright, prop-like cells which are upto 16 μ in height and 6 μ in diameter. The central portion of the perithecial cavity is occupied by the asci and paraphyses (fig. 12).

The asci are paraphysate and arise from ascogenous hyphae round the outer edge of the ring of the palisade-like prop-cells. The cells of the ascogenous hyphae give rise to croziers which ultimately develop into cylindrical-clavate asci, tapering at both ends and thickened at the apex. The asci are 8-spored, obliquely horizontal, converging towards the ostiole and measure 36–40 × 6.5–8 μ. The ascospores are 2–3 seriate, cylindrical-spindle-shaped, hyaline, bicelled and 12–15 × 2–2.5 μ in size. The upper cell of the ascospore is slightly shorter and broader, 6-ciliate, but of the six cilia, three project out at the top and the other three at the sides. The cilia arise from a central point in the upper cell of the ascospore and when pressed out, appear to be three continuous filaments. The projecting portions of the three apical cilia are 8–10 μ in length and the three lower ones extend from 15 to 20μ. The paraphysoids are numerous, thin, hyaline and filiform. The central portion of the thyrothecium, below the asci, is filled up with a broad, triangular column of paraphysoidal threads which, later on, tends to disintegrate.

The morphology of the fungus indicates that it belongs to the genus *Actinopeltis* of the family *Trichothyriaceae*, which is characterised by the presence of bristles round the ostiole. The characteristic prop-like cell in the thyrothecium and the peculiar cilia, clearly distinguish the fungus on *Quercus glauca* from the other species of the genus so far recorded.

13. *Meliola melanochaeta* Syd.—Ann. Mycol., **26**: 93 (1928)

Material examined: On living leaves of *Quercus leucotrichophora* A. Camus, collected in Ranikhet, on 18.7.1961, 9.10.1962, 28.12.1962, leg. S.K. Bose, Pauri, Gadoli, Pauri Garhwal, on 30.8.1962, leg. A.J. Roy.

The fungus is parasitic on the upper surface of living leaves and forms scattered, round, black spots, which are upto 0.5 μ in diameter. The mycelium is composed of dark brown, septate, branched hyphae, measuring 7–10 μ in diameter. Both mucronate and capitate hyphopodia are present. The mucronate hyphopodia are usually opposite, sometimes solitary, unicellular and measure 15–25 × 8–12 μ. The capitate hyphopodia are usually alternate, bicelled, the lower cell being 6–10 μ in diameter and the capitate cell 10–16 μ in diameter. The branches and the hyphopodia often anastomose. Mycelial setae, measuring 240–480 × 7–9 μ, are also present, both on the hyphae and round the base of the perithecia. The setae are bluntly pointed or forked at the apex (fig. 13).

The ascocarps are scattered, globose, slightly flattened at the base and 100–200 μ in diameter. The wall of the ascocarp is composed of a single layer of brown cells, measuring 6–14 μ in diameter, wh

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

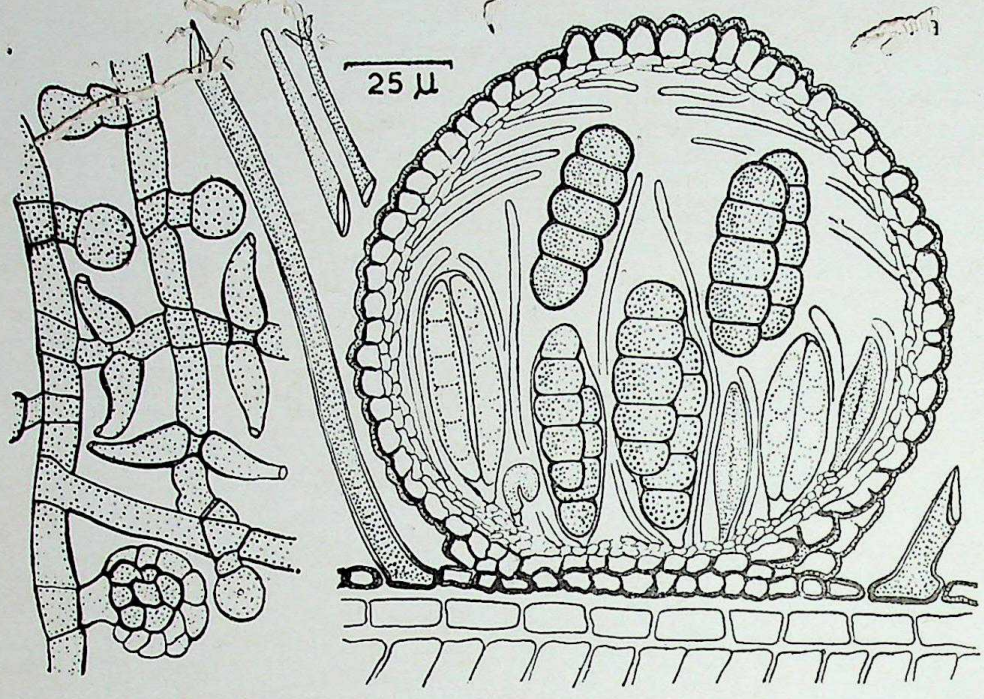


Fig. 13. *Meliola melanochaeta.* Section through an ascocarp, 500×. Left—Mycelium with hyphopodia and young ascocarp, 500 ×.

are thick-walled on the exposed surface. The flattened base consists of two or more layers of thick-walled cells. The ascocarp cavity is lined with two or more layers of elongated, hyaline, pseudoparenchymatous cells.

The asci are sessile, elongated oval or fusiform, usually two spored, but in rare cases one or even three spored asci have been observed. In early stages, hyaline paraphysoids are present, but later on, these become evanescent along with the ascus wall. The ascospores are brown, 4-septate, constricted at septa, rounded at the ends and measure 35—50 × 14—20 μ.

Although the ascospores are slightly smaller, the fungus closely resembles *Meliola melanochaeta* which occurs in Borneo on *Quercus spicata* Sm., and is considered to be identical.

14. *Phacidium kumaonensis* nov. spec.

Ascomata epiphylla, dispersa, in epidermidem nascentia, lentiformia, rotundata vel plusminusve quadrata, 500—900μ latitudine, 200—240μ altitudine, atro-brunnea, primo aperta, laciniis irregulariter dehiscentia, 18—24μ crassitudine composito cellulis rotundatis, fuscis, 3—6μ diam. Paries basalis 15—22μ crassam cellulis subfuscis vel hyalinis composita. Asci unitunicati, cylindraceo-clavati, plusminusve stipitati, dense aggregati, 60—145 × 9—13μ, 8-spori; paraphyses filiformes, ascomata matura pitvitosa. Sporae unicellulatae, elongato-ellipsoideae, saepe leniter curvatae, hyalinae, 11—15 × 4—4.5μ.

Forma conidiophora similis ; pycnidia 200—300 × 50—60μ magnitudine, pars interior hyphis dense aggregatis filiformibus composita.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Hyphae posterius septatae, fragmenta fiunt et conidias unicellulatas, hyalinas, $2-3 \times 1-2\mu$ magnitudine formant.

Hab. in foliis vivis *Querci leucotrichophorae* A. Camus (=*Querci incani* Roxb.), Kumaon, Ranikhet (Dhobi Ghat), 19.10.1962, leg: T. C. Sharma.

The lens-shaped, epidermal, black ascomata appear scattered on the upper surface of the dead leaves of *Quercus leucotrichophora* and measure $500-900 \times 200-240\mu$. The upper wall is $18-24\mu$ in thickness and is composed of several layers of brown, polyhedral cells, which are $3-6\mu$ in diameter, The basal wall of the ascomata is $15-22\mu$ in thickness and is composed of hyaline pseudoparenchymatous cells, measuring $2-4\mu$ in diameter. The upper and lower walls are closely knit at the edges by brown, polyhedral cells.

The club-shaped, unitunicate asci are closely packed with their upper ends standing at various levels and their stipes of different length forming a compact parallel mass at the base. The asci are $60-145 \times 9-13\mu$ in size. The young asci are paraphysate in early stages, but the paraphyses soon become evanescent due to subsequent compression by the maturing asci. The ascospores are eight in number, hyaline elongated-ellipsoid, straight or slightly curved, sometimes tapering at the lower end and measure $11-15 \times 4-4.5\mu$. The ascospores are obliquely uniseriate in the longer asci, but tend to become irregularly biseriate or crowded within the smaller ones (fig. 14).

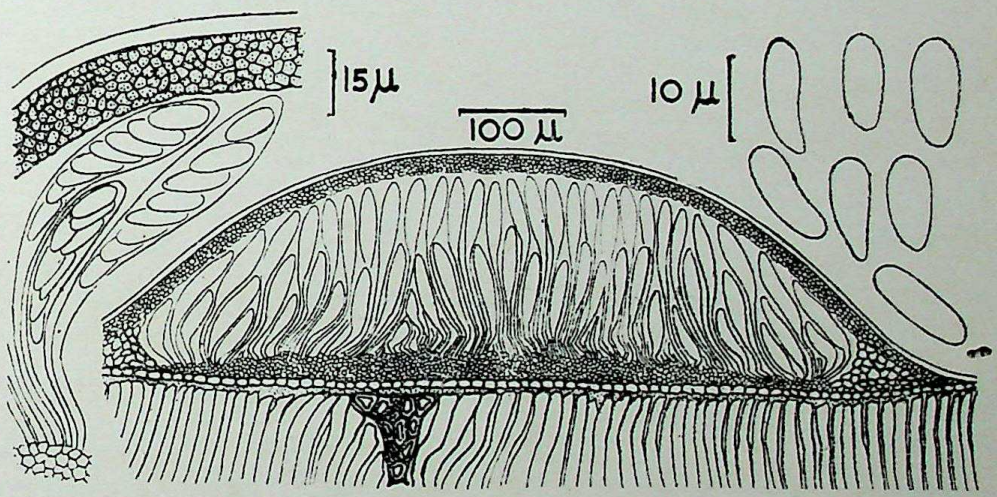


Fig. 14. *Phacidium kumaonensis*. Section through a fruit body 250×. Left—Asci, 500×. Right—Ascospores, 1,000×.

The broken imperfect stage of this fungus has been found to occur mixed with the ascocarps and is similar to the latter in outer appearance. But these are smaller, not more than 300μ in diameter and, therefore, can be easily recognised. These are also lens-like in shape and $50-60\mu$ in height. The wall is dark brown, $6-8\mu$ in thickness and formed of $1-2$ layers of thick-walled, more or less spherical cells, measuring $4-8\mu$ in diameter. The wall is without any ostiole at the top. The basal

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

part of the fructification is 6—10μ in thickness and composed of approximately spherical, brown cells, measuring 1—2μ in diameter, which fill the epidermal cells. In the beginning, the cavity is full of a thick network of vertically growing hyphae, measuring 1—2μ in diameter. Later on, the network divides, especially in the upper parts, into segments which are 2—3μ in length, forming oidia like spores, measuring 2—3 × 1—2μ in size. In the centre, the individual hyphal bundles remain unchanged and, therefore, appear to be divided into very few locules. The imperfect stage belongs to the form-genus *Ceuthospora*.

The fungus, without doubt, belongs to the genus *Phacidium* Fr., although it exhibits a number of peculiarities by which this species departs from the type of the genus *Phacidium lacerum* Fr., somewhat strongly. The wall remains closed for a long time, the asci have stipes of variable lengths and therefore stand, as is often the case with *Pseudophacidium ledi* (Alb. et Schw.) Karst., at different heights and also, as is the case with this branch and stem inhabiting species, the paraphyses are usually absorbed in the ripe ascomata.

Summary

A number of fungi from central Himalayas have been investigated. The fungi described in this paper are *Ferrarisia quercina* sp. nov. *Lembosia decolorans* Syd., *Johansonia arxi* sp. nov., *Sphaerulina quercifolia* sp. nov., *Coccoidella quercifolia* sp. nov., *Dimerium venturioides* (Sacc. et Berl.) Theiss., *Wentiomyces javanicus* Koorders, *Coccochora kusanoi* (P. Henn.) v. Höhn., *Microthyrium biciliatum* sp. nov., *Microthyrium pieridis* sp. nov., *Micropeltis congonensis* Beeli, *Actinopeltis ciliaris* sp. nov., *Meliola melanochaeta* Syd., and *Phacidium kumaonensis* sp. nov.

Hill Fruit Research Station, Chaubattia,
U.P., and Institute of Special Botany,
Swiss Federal Institute of Technology,
Zürich, Switzerland.

References

HANSFORD, C.G. (1946) The foliicolous Ascomycetes, their parasites and associated fungi. *Imp. Myc. Inst., Mycological paper* no. 15.

HENNINGS, P. (1904) Fungi fluminensis a cl. E. Ule. collecti. *Hedwigia*, **43** : 78-95.

HÖHNEL, F. VON (1910) Fragmente zur Mykologie, Nrn. 407-467. *Sitzungsber. K. Ak, Wiss. Wien, math. nat.* Kl., 1, Abt., **118** : 1461-1552.

MULLER, E. (1954) Beitrage zur Kenntnis der Gättung *Gibbera* Fr. emend Petr., *Sydowia*, **8** : 60-73.

MÜLLER, E., AND J. A. VON ARX (1962) Die Gattungen der didymosporen Pyrenomyceten. *Beitr. Krypt. Flora Schweiz*, Band **11** : no 2, 922 pp.

THEISSEN, F., AND H. SYDOW (1915) Die Dothideales. Kritischsystematische Originaluntersuchungen. *Ann. Mycol.*, Berl. **13** : 149-746.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON FOUR ISOLATES OF RHIZOCTONIA SOLANI KUEHN, THE CAUSAL AGENT OF BLACK SCURF DISEASE OF POTATO IN THE PUNJAB—I

SYMPTOMS, STRAINS AND PATHOGENICITY

MADHO SINGH

(Accepted for publication September 14, 1963)

INTRODUCTION: The black scurf disease of potato (*Rhizoctonia solani* Kühn) has been gaining prominence in the Punjab State for the last few years. Thirumalachar (1953) reported that *R. solani* occurs on potato both in the plains and hills of India.

The seed tubers bearing the sclerotia of the fungus, when sown in the soil, give rise to infected sprouts, which either fail to come out because of their pre-emergence wilt or if they escape the early onset of the disease, emerge from the soil but may be subsequently attacked by the fungus which creeps up and produces brown, sunken, elongated lesions on the stems. The spots may girdle the stem so as to cause premature wilting or death of the plant. The stolons are likewise attacked and may fail to produce any tubers. Richter and Schneider (1951) reported that *Rhizoctonia* infections may be most extensive on the stolons and the loss may amount to more than 70 per cent in certain cases. Another type of injury, was the production of "aerial tubers" by the buds towards the base of the stem. This phase of the disease was found to be especially common in the late autumn sowings.

The plants in which infections become more or less established, produce sclerotia on the tubers. These are usually irregular in shape and black in colour and adhere closely to the skin of the tuber. They vary in size from that of a pea seed to that of a pin-head or are even smaller. They often coalesce at various places on the tuber surface. The disease was found to be much more severe in the autumn crop than in the spring crop. The sclerotia were much less abundant and smaller in size in the latter case.

EXPERIMENTAL: Isolations were made from a large number of potato tubers collected from various cold stores in the Punjab State. All the cultures were purified by single-hyphal tip culture method. The purified isolates could be grouped into four main categories on the basis of their cultural characters. The three sclerotial isolates were named as A, B and C (Fig. 2), and the mycelial isolate as D. Their main morphological differentiating characters when grown on nutrient dextrose broth, are given in Table 1. Isolate A produces scattered sclerotia, whereas, isolates B and C produce them in the form of concentric rings and crust respectively. Isolate D does not produce any sclerotia.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Important cultural characters of the four isolates of *R. solani* on nutrient glucose agar, as recorded after 25 days at room temperature (20° to 26°C.)

Name of isolate	Average colony diameter (mm.) after 4 days	Arrangement of sclerotia on the surface of the medium	Colour of sclerotia
A	53	Scattered, almost all over the surface. Bigger sclerotia present towards the centre	Brown
B	43	Disposed in 2 or 3 concentric rings	Deep brown
C	14	In the form of a crust covered over with dense mycelium	Brownish black
D	80	Only very minute sclerotial initials formed	Sclerotial initials white or light yellow brown in colour

Pathogenicity tests were conducted in wooden flats containing sterilized soil. Mass cultures of the four isolates were produced in conical flasks containing autoclaved carrot slices. For inoculation with each isolate, in 4 wooden flats a weighed quantity of the mass culture was thoroughly mixed with the soil in each flat. 4 similar flats were kept uninfested to serve as control. 8 disease-free seed tubers treated with 0.1 per cent mercuric chloride solution, were sown in each flat. At the end of the growing season, the plants with the tubers were carefully dug out with intact roots and stolons and washed with tap water for examination. The results of the pathogenicity tests are set out in Table 2 and also represented in Fig. 1.

It may be seen from the data given in Table 2 that the mycelial isolate D is non-pathogenic, while the isolates A,B and C are highly pathogenic but differing from one another in their pathogenic effect. Isolate A appears to be more virulent than B and C which do not differ much among themselves. From figure 2, it may be seen that the isolate A in addition to producing sclerotia on the surface of the tubers, causes russettng and corrosion of the skin. Isolate B, produces well-developed sclerotia and only very slight corrosion and russeting on the skin, while isolate C does not produce such effects and forms sclerotia which are very thin and flat. Isolations from the parts of the plants affected with the different isolates, yielded the same isolate, which was used to inoculate the plants.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Pathogenicity tests of the four isolates of *R. solani*

Isolate	Total number of tubers sown in inoculated soil	Per cent pre- and post-emergence wilt	Percentage of sprouts showing infection	Total number of stolons showing infection	Percentage of 'scurfed' tubers
A	32	26.3	52.6	109	89.7
B	32	16.7	54.2	98	86.7
C	32	10.8	71.1	74	71.8
D	32	0	0	0	0
Uninoculated (Control)	32	0	0	0	0

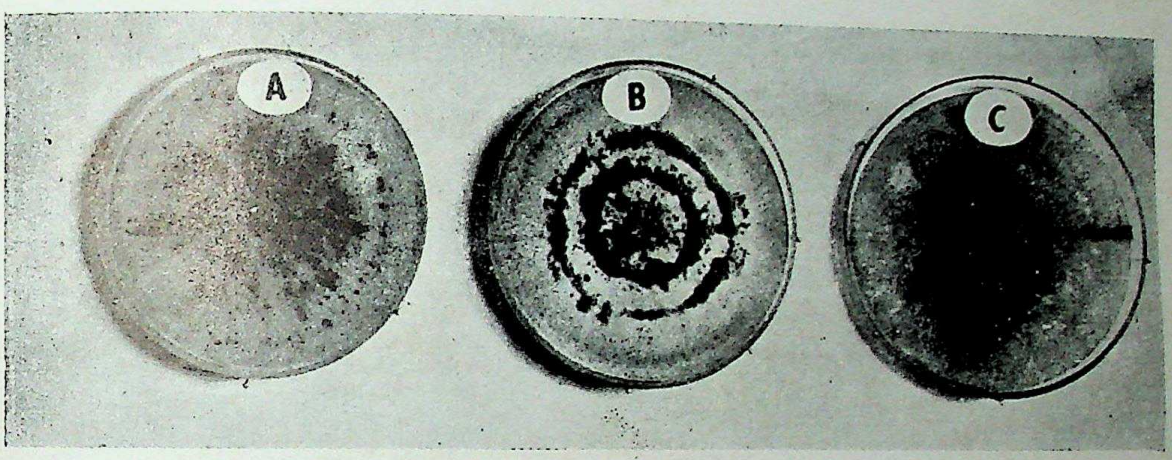


Fig. 1. Three cultural isolates of *R. solani*

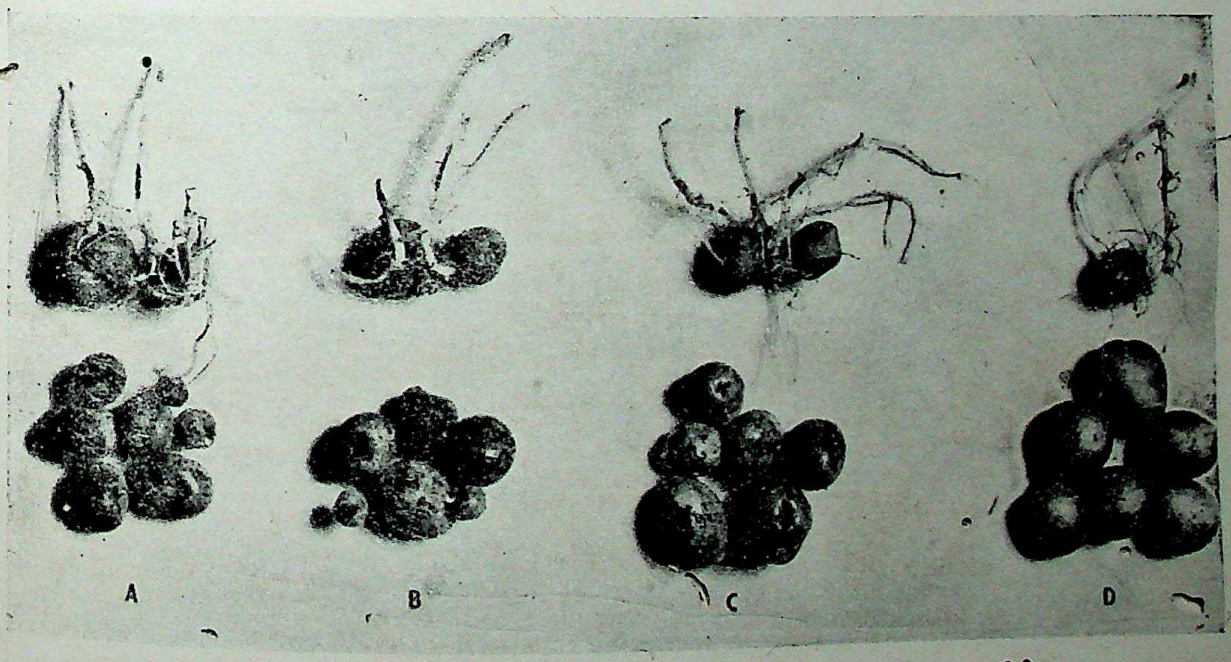


Fig. 2. (A) Sprouts and tubers affected with the isolate A. (B) Sprouts and tubers affected with the isolate B. (C) Sprouts and tubers affected with the isolate C. (D) Sprouts and tubers from soil infested with isolate D showing no infection

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

These results are in conformity with those obtained by Person (1945) who reported that 17 out of 70 isolates of *R. solani* from potatoes were non-pathogenic, 35 were slightly pathogenic, and only 5 were strongly pathogenic.

SUMMARY

The black scurf disease of potato which occurs quite commonly in the Punjab State, has been investigated. The black sclerotial encrustations adhere closely to the skins of potato tubers which, when sown may give rise to infected plants. There may be pre-emergence or post-emergence wilt. The plants which recover from these phases, produce tubers bearing the sclerotia. Isolations made from sclerotia on the tubers collected from different localities and their subsequent purification resulted in the production of four isolates exhibiting clear morphological and pathogenic differences.

ACKNOWLEDGEMENTS : My grateful thanks are due to Dr. K. S. Bedi, Ex-Joint Director of Agriculture (R & E). Punjab, for suggesting the problem and valuable guidance throughout the course of these investigations.

Plant Pathological Section,
College of Agriculture,
Punjab Agricultural University,
Ludhiana.

REFERENCES

PERSON, L. H. (1945) Pathogenicity of isolates of *R. solani* from potatoes. *Phytopathology*, **35** : 132-134.

RICHTER, H. and Schneider, R. (1951) *Rhizoctonia* injuries on potato stolons, roots and tubers and sprouts. *Phytopathology*, **17** : 374-383.

THIRUMALACHAR, M. J. (1953) *Rhizoctonia solani* infection of potato tubers in India. *Phytopathology*, **43** : 645-647.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ON THE PHYSIOLOGY OF ASCOSPORE FORMATION IN POWDERY MILDEW OF WHEAT

H. C. ARYA

(Accepted for publication October 10, 1963)

The formation of ascospores in the cleistothecium of *Erysiphe graminis* var. *tritici* El. Marchal, (powdery mildew of wheat) has been considered important for its possible role in the perpetuation and recurrence of the disease (Salmon, 1903; Yossifovitch, 1929; Cherewick. 1944; Thalmann, 1950; Arya and Ghemawat, 1953; and Moseman and Powers, 1957). A general survey of the incidence of the disease in Rajasthan revealed that the mildew attacked the wheat crop in the low lying areas of Jodhpur, Udaipur, and Bharatpur. In these areas, the oidial stage was quite common, but the cleistothecial formation though not so common, was present late in the season. In the cleistothecium, asci were present, but the ascospore formation was lacking. Certain experiments were conducted to explore the possibility of ascospore formation under various sets of physical and chemical influences. The results are presented here.

MATERIAL AND METHODS : The infected wheat leaves with cleistothecia were collected from the Umaid Sagar area of Jodhpur. The cleistothecial material was also obtained from the fungus maintained on the Jodhpur local wheat (an indigenous or 'desi' variety of *Triticum aestivim* L.) in the laboratory.

Two methods were employed : (1) cleistothecia were removed from the host tissue and were used for various treatments; (2) wheat leaf with immature cleistothecia (without ascospores) was floated with its leaf sheath dipped in 10.0 per cent sucrose solution or in distilled water (Yarwood, 1957), and subjected to different conditions. Sterilized nutrient solution in the treatment was changed every 24 hr. in order to maintain the desired concentrati n. Individual experiments were given 6 replications and each experiment was repeated 3-4 times to confirm the results.

EXPERIMENTAL RESULTS : *Effect of temperature* : 2″ pieces of wheat leaves with cleistothecia containing asci but no ascospores were floated on sterilized distilled water with their leaf sheaths dipped in the solution, and were incubated at 0, 5, and 9°C for different intervals of time. The cultures were afterwards maintained at 24±2°C. The results are presented in Tables 1, 2 and 3.

It is evident from the data given that there was ascospore formation after freezing the material at 0, 5 and 9°C for 8-10 hr. and exposing it to 24±2°C for various periods. However, the percentage of ascospore formation in the treatments was not uniform. This may be due to variations in the ages of the cleistothecia examined.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results obtained are in confirmity with those recorded by Graf-Marin (1934) in the case of *Erysiphe graminis* var. *hordei*, who reported ascospore formation after freezing the material at 9°C and exposing it to 21°C for 22 hr.

TABLE 1 : Ascospore formation at 0°C

Exposure in hr. at 24°C	Exposure to low temperature of 0°C		
	4 hr.	8 hr.	10 hr.
	per cent ascospore-forming cleistothecia		
12	0.0	0.0	0.0
24	0.0	0.0	10.0
36	0.0	15.0	20.0
48	0.0	25.0	25.0
72	0.0	40.0	45.0
96	0.0	40.0	45.0
120	0.0	60.0	65.0

TABLE 2 : Ascospore formation at 5°C

Exposure in hr. at 24°C	Exposure to low temperature of 5°C		
	per cent ascospore-forming cleistothecia		
12	0.0	0.0	0.0
24	0.0	0.0	10.0
36	0.0	10.0	15.0
48	0.0	20.0	20.0
72	0.0	25.0	30.0
96	0.0	40.0	35.0
120	0.0	60.0	60.0

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Ascospore formation at 9°C

Exposure in hr. at 24°C	Exposure to low temperature of 9°C		
	4 hr.	8 hr.	10 hr,
	per cent ascospore-forming cleistothecia		
12	0.0	0.0	0.0
24	0.0	0.0	15.0
36	0.0	10.0	20.0
48	0.0	20.0	20.0
72	0.0	30.0	25.0
96	0.0	50.0	40.0
120	0.0	60.0	60.0

2. *Effect of sucrose* : Different concentrations of sucrose ranging from 2-25 per cent were prepared. The infected leaves with cleistothecia were floated as usual on these solutions for different periods at 24°C. The results obtained are recorded in Table 4. The data show that there was better ascospore formation at the sugar concentrations from 5-25 per cent, after an exposure of 120 hr.

TABLE 4 : Ascospore formation in different sucrose concentrations

Exposure in hr. at 24°C	per cent ascospore forming cleistothecia in sucrose concentrations of						
	2%	5%	8%	10%	15%	20%	25%
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0	0.0	8.0
24	0.0	0.0	0.0	0.0	5.0	10.0	20.0
36	0.0	0.0	0.0	10.0	15.0	20.0	30.0
48	0.0	0.0	0.0	20.0	20.0	30.0	35.0
72	0.0	0.0	0.0	25.0	25.0	35.0	40.0
96	0.0	10.0	10.0	25.0	25.0	35.0	40.0
120	0.0	15.0	15.0	25.0	25.0	35.0	40.0

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

3. *Effect of nitric acid* : Different dilutions were obtained by using various fractions of a millilitre of concentrated nitric acid (69.0 per cent) dissolved in 5 ml. of sterilized distilled water. The cleistothecial material was subjected to these dilutions for different periods at 24°C. The positive results, showing ascospore formation, were obtained at concentrations of 0.3, 0.4, and 0.5 ml. nitric acid in 5 ml. of sterilized distilled water after an exposure of 120, 144, 168 and 192 hr.

4. *Effect of potassium nitrate* : The cleistothecial material was subjected to different potassium nitrate dilutions ranging from 8-30 per cent for different periods at 24°C. There were indications of only segmentations of cytoplasmic contents and not ascospore differentiation. With 25 per cent potassium nitrate solution segmentation was initiated after an exposure for 168 hr. and with 30 per cent potassium nitrate solution it was obtained after an exposure for 120 hr.

5. *Effect of vitamins* : Concentrations of the vitamins thiamine hydrochloride, lactoflavin, nicotinic acid, biotin, i-inositol, and pyridoxine, were prepared in sterilized distilled water. The dosages used were the same as recommended by Lilly and Barnett (1951) with suitable modifications.

The infected wheat leaves with cleistothecia were floated as usual on these solutions for varying periods at 24°C. The results are recorded in Table 5.

TABLE 5 : Effect of vitamins on ascospore formation

Vitamins p. p. m.	Per cent ascospore-forming cleistothecia at hr.						
	12	24	36	48	72	96	120
Thiamine 500	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactoflavin 500	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nicotinic acid 500	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biotin 50	0.0	0.0	0.0	10.0	15.0	15.0	20.0
Inositol 50	0.0	0.0	20.0	35.0	40.0	50.0	60.0
Pyridoxine 500	0.0	0.0	0.0	10.0	15.0	25.0	35.0
Dist. water	0.0	0.0	0.0	0.0	0.0	0.0	0.0

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The data show that vitamins, thiamine hydrochloride, lactoflavin and nicotinic acid had no effect on the ascospore formation while vitamins biotin, inositol, and pyridoxine gave positive results. The results were in contrast to those reported earlier (Arya and Ghemawat, 1953).

6. *Effect of alternate wetting and drying* : Cleistothecial material was subjected to different periods of alternate wet and dry conditions at 18, 22. and 27°C; the cleistothecia which were collected in the previous season were soaked in water for different periods and then dried at the above temperatures separately for different timings and afterwards transferred to moist filter papers and examined. In the last treatment the cleistothecia often burst to release the ascospores. The results are presented in Table 6.

TABLE 6. Effect of alternate wetting and drying on ascospore formation

Alternate periods in hr.	Per cent ascospore formation at		
	18°C	22°C	27°C
12	0.0	0.0	0.0
24	0.0	8.0	15.0
48	0.0	15.0	25.0
72	0.0	20.0	35.0

The data show that alternate wetting and drying have a positive effect on the ascospore formation at 22°C and 27°C. Similar results have been recorded by Cherewick (1944) under Canadian conditions.

7. *Effect of different soil conditions* : The cleistothecial material, immediately after collection from the field, was kept wrapped in cellophane paper bags and buried in earthen pots filled with soil at a depth of 2-3 inches. They were incubated under the following conditions: (1) in the sun with or without wet soil, (2) in the shade with or without wet soil, and (3) alternate dry and wet soil conditions with alternating sun and shade situations.

In the last treatment the soil was kept dry during April to June and moist during the months of August and September and then dry again with occasional watering upto April. The soil temperature was recorded every day. The experiments lasted for twelve months from April 1957 to April 1958. Fortnightly readings on the ascospore formation were taken by examining cleistothecia kept under different treatments. The results were negative in the case of first four treatments. However, the last treatment gave positive results and they are presented in Table 7.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 7 : Effect of alternate dry and wet soil conditions on ascospore formation

Observation in the months	Total number of cleistothecia		
	Examined	Fertile	Per cent fertility
November	50	0	0.0
December	50	0	0.0
January	50	0	0.0
February	50	20	40.0
March	50	30	60.0
April	50	30	60.0

It is clear that from February onward ascospores started forming in the cleistothecia.

DISCUSSION : The role of cleistothecia in the annual recurrence of the powdery mildew disease of wheat has been a subject of much controversy (Bioletti, 1907; Brooks, 1928; Mehta, 1930; Savulescu *et al.*, 1935; and Sempio and Castori, 1949). Honecker (1936) expressed the view that the cleistothecia played an important role in the spread of the disease. Foster and Henry (1937) studying the annual recurrence of the disease in Alberta, found that the conidia are not concerned in the overwintering of the fungus but it is at the cleistothecial stage that the fungus overwintered. Cherewick (1944) found that the cleistothecia of *E graminis hordei* and also that of *E graminis tritici* were without ascospores. According to him ascospore formation could be induced by subjecting the cleistothecia to wet and dry conditions alternately. By following this method he could establish fresh cultures of the powdery mildews and could keep the cleistothecia viable for three years in the refrigerator.

From the results obtained it may be concluded that, (1) the cleistothecia produced on the crop are not permanently sterile. Once they are treated or subjected to a certain set of conditions, they become fertile and ascospores are formed in the asci, (2) ascospore formation could be secured in the laboratory under the conditions analogous to those obtainable in nature.

It will be interesting to describe in brief the conditions to which the fields where severe infection occurs every year, are subjected. After harvest dry weather follows for two or three months (April, May and June). This is followed by rainy season when the area gets submerged under water for two to three months (August, September, and October).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

After water has receded the fields are prepared and sown with wheat. During the third or fourth week of February infection starts from the lowest leaves of the plants ; these leaves are naturally very near the soil surface and some of them often touching it.

The results obtained under laboratory conditions lead to a logical presumption that the cleistothecial material found in nature may be responsible for the initiation of infection every year in the fields.

SUMMARY

Physiology of ascospore formation in the cleistothecium has been studied by two methods : 1) cleistothecia were removed from the host tissue and were used for the experimental treatments, and (2) wheat leaf with immature cleistothecia (without ascospores) was floated with its leaf sheaths dipped in 10.0 per cent sucrose solution or in distilled water and subjected to different treatments.

Cleistothecia on the wheat leaves, when subjected to low temperatures of 0, 5. and 9°C, showed formation of ascospores to the extent of 60-65 per cent.

Sucrose concentrations ranging from 5-25 per cent, nitric acid concentrations ranging from 0.3 to 0.5 ml. per 5 ml. of water and different vitamins like biotin, inositol and pyridoxine gave positive results in the ascospore formation in the cleistothecium.

When cleistothecial material was subjected to different periods of alternate wet and dry conditions at 18°, 22°, and 27°C, ascospore formation was obtained at 22° and 27°C. When cleistothecial material was subjected to alternate dry and wet soil conditions for 12 months from April, ascospore formation was obtained after an interval of about ten months.

ACKNOWLEDGEMENT : The author wishes to express his thanks to Professor S. N. Das Gupta, Vice-Chancellor, Kalyani University, Calcutta, for his keen interest during the course of this work.

Department of Botany,
University of Rajasthan,
Jaipur.

REFERENCES

ARYA, H. C. AND M. S. GHEMAWAT, (1953) Occurrence of powdery mildew of wheat in the neighbourhood of Jodhpur. *Indian Phytopath.* **6** : 123-130.

BIOLETTI, F. T. (1907) *Oidium* or powdery mildew of the vine. *Calif. Agric. Expt. Sta. Bull.* **186** : 315-350.

BROOKS, F. T. (1928) *Plant diseases*, Oxford University Press.

CHEREWICK, W. J. (1944) Studies on the biology of *Erysiphe graminis* DC. *Can. J. Res.* (C) **22** : 52-86.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FOSTER, W. R. AND A. W. HENRY (1937) Overwintering of certain cereal pathogens in Alberta. *Can. J. Res.* (C) **15** : 547-559.

GRAF-MARIN, A. (1934) Studies on the powdery mildew of cereals. *Cornell Univ. Agric.Expt. Sta. Mem.* 157.

HONECKER, L. (1936) Uber den derzeitigen stund und die Aussiehten der Bekampfung des Meltaubefallus der Gerste durch Zuchtung, *pract. Bl. Pflazenb.* 12 : 309-320.

LILLY, V. G. AND H. L. BARNETT (1951) *Physiology of the fungi.* McGraw Hill Book Co., New York.

MEHTA, K. C. (1930) Studies on the annual recurrence of powdery mildews of wheat and barley in India. *Agric. J. India*, **25** : 283-285.

MOSEMAN, J. G. AND H. R. POWERS (1957) Function and longevity of cleistothecia *Erysiphe graminis* f. sp. *hordei. Phytopathology*, **47** : 53-56.

SALMON, E. S. (1903) Infection powers of ascospores in Erysiphaceae. *J. Bot.* **41** : 159-165.

SAVULESCU, T. C., SANDU-VILLE, T. RAYSS, AND V. ALEXANDRI (1935) L'e tat phytosanitative en Roumaine an cours de L'anne 1933-34. *Inst. Cere. Agron. al Romanei.*, **24** : 59.

SEMPIO, C. AND M. CASTORI (1949) Extrema sensibilita dei conidi di Oidium monilioides per tracee di vaporidl zolfo. *Riv. Biol. N. S.*, **41** : 163-172.

THALMANN, L. (1950) Formazione di aschi nei periteci di *E. grami- nis* in vitro, in presenza di vitamine, fitormoni ed estratti vegetali. Notiz Malatt. Piante (*Rev. Appl. Mycol.*, **29** : 462, 1950).

YARWOOD, C. E. (1957) Powdery mildews. *Bot. Rev.*, **23** : 235-278.

YOSSIFOVITCH, M. (1929) Le mecanisme de la separation des Erysiphacees et le role des fulcres. *Rev. Path. Veg. et Ent. Agr.*, **16** : 132-140.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ADAPTATION OF SCLEROTIUM ROLFSII TO COPPER COMPOUNDS[1]

D. V. RAGHAVA REDDY[2] AND A. APPARAO

(Accepted for publication December 15, 1963)

INTRODUCTION : Adaptation of fungi to toxic chemicals *in vitro* has been the subject of a number of investigations during recent years (Christensen and Daly, 1942 ; Stakman, 1946 ; Gattani, 1951 ; Buxton, 1960 ; Grover and Moore, 1961). Reports of the occurrence of resistant strains of fungi *in vivo* are, however, rare. Taylor (1952) observed that the spores of the apple black rot fungus *Physalospora obtusa*, from orchards regularly sprayed with Bordeaux mixture, developed more resistance to copper than the spores from unsprayed orchards. Reduction in the effectiveness of Bordeaux mixture to control late blight of potatoes caused by *Phytophthora infestans* after its use for 60 odd years has been atributed by Horsfall (1956) to increased tolerance of the fungus to Bordeaux mixture. Such resistance *in vivo* is also likely to develop in the soil-borne pathogens if repeated application of fungicides against them by drenching the soil or spraying at the base of the plant is made. In the present investigation a soil borne fungus, *Sclerotium rolfsii*, causing wilt of chillies and other economic plants, has been tested for its capacity to develop increased tolerance to copper compounds *in vitro*.

MATERIAL AND METHODS : A culture of *Sclerotium rolfsii* originally isolated from chillies was employed. The toxic chemicals used were $CuSO_4.5H_2O$ and Cheshunt compound. Cheshunt compound was prepared by mixing two parts by weight $CuSO_4.5H_2O$ and 11 parts of thoroughly grounded ammonium carbonate. The mixture was kept in an airtight bottle for 48 hours to ripen and the ripening period was kept constant throughout the experiments. The fungicides were incorporated on the basis of their copper content to 25 ml. of sucrose-nitrate medium in 250 ml. flasks and all concentrations are expressed as amounts of copper per flask. 'AnalaR' grade chemicals were used. Trace element and vitamin mixtures were supplemented to the basal medium. Two per cent agar cleaned of all foreign matter by washing in water was used for studies on solid medium. All the cultures were incubated at room temperature (24°—28°C) in glass culture chambers for fifteen days.

Sclerotial formation was qualitatively assessed by adopting the following ratings : O=no sclerotia, +=10 per flask, ++=10-20 per flask, +++ = 20-30 per flask, ++++ = 30-50 per flask, abundant = above 50 per flask.

---

[1]Part of the thesis submitted by the senior author for in partial fulfilment requirements of M.Sc. (Ag.) degree.

[2]Presently at the Department of Botany, University of Illinois, Urbana, Ill., U.S.A.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL RESULTS : *Adaptation of* S. rolfsii *to Cheshunt compound* :—(a) Liquid medium :

An initial experiment with the parent strain of *S. rolfsii* revealed the highest concentration of the fungicide allowing the growth of the fungus to be 1000 μg. of copper in the form of cheshunt compound.

The fungus was grown in concentrations ranging from 500 μg. to 3000μg. for twelve generations, the inoculum, each time, being taken from the previous generation. At the first subculturing itself the fungus could tolerate 2000μg. of copper in the form of Cheshunt compound (Table 1). No increased tolerance was observed until 9th generation when growth occured at a concentration of 2500 μg. In the 12th generation the fungus could tolerate a concentration of 3000 μg.

TABLE 1 : Growth of *S. rolfsii* on media containing various amounts of copper in the form of cheshunt compound

Fungicide μg. of copper	Growth in successive generations in mg.*												
	Initial tolerance	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
500	306												
1000	249	389											
1500	0	388	346	401	396	385	412	389	389	396	385	415	445
2000	0	383	347	380	409	381	402	383	399	374	347	385	394
2500	0	0	0	0	0	0	0	0	0	209	234	228	385
3000	0	0	0	0	0	0	0	0	0	0	0	0	274

*The levels against which no figures appear are not tested in the respective generations.

(b) Solid medium :

The basal medium was solidified with 2 per cent cleaned agar and 20 ml. of the medium was taken for each petriplate. The concentration of the copper used ranged from 1000 to 5000 μg. as given in Table 2. The inoculum for study was taken from fungal colonies grown on 1500 μg. of copper of the 9th generation. The fungus grew even in 5000 μg. of copper, in the presence of agar in the medium, but the growth was comparatively slower and peculiar in that it was raised in concentric circles nearly touching the lid of the petriplate. It might be mentioned that in liquid cultures the fungus could tolerate concentration of 2500 μg. only in the 9th generation. Parry and Wood (1958) reported that *Botrytis cinerea* tolerated higher amounts of copper in solid medium than in liquid medium.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Growth and sclerotial formation of *S. rolfsii* sucrose-nitrate agar containing various amounts of copper in the form of Cheshunt compound

Sl. No.	Conc. of fungicide in μg. of copper.	Average linear growth in mm. Days 2	4	6	8	10	12	14	Sclerotial ratings
1.	5000	2	9	26	34	43			+
2.	4000	2	12	29	38	45			+++
3.	2000	5	16	39	45				Abundant
4.	1000	5	16	40	45				Abundant
5.	Control	6	22	43					Abundant

TABLE 3 : Growth of *S. rolfsii* on media containing various amounts of copper in the form of copper sulphate

Fungicide μg. of copper	Growth in mg. in successive generations* Initial tolerance	1st	2nd	3rd	4th	5th	6th**	7th	8th	9th	10th	11th	12th
1000	384	460											
3000	381	452											
4000	386	458	423										
5000	388		392										
6000	358		419	323									
7500	261			318	323	314							
9500	103			209	262	231		293	400				
10000	0			192	248	209			271	411			
12500					149	148		227	376	427			
13500						88		189	391	402			
14000						0		165	385	401	434		
14500						0		115	360	356	436		
15000								0	349	356	428		
15500								0	351	348	417		
20000										285	449	483	504
30000											404	518	501
40000												458	483
50000												431	545
60000													481
70000													502
80000													157
85000													0

*The levels against which no figures appear were not tested in the respective generations.

**The series of experiment failed due to inexplicable reasons.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

2. *Adaptation of* S. rolfsii *to copper-sulphate* :

Maximum concentration of the fungicide tolerated in liquid medium by the parent strain of *S. rolfsii* was determined to be 9500 μg. of copper. This parent strain was grown in media incorporated with increasing amounts of fungicide for twelve successive generations distributed over a period of 6 months. By such gradual training process, a strain of *S. rolfsii* was obtained which could tolerate 80,000 μg. of copper though growth was slow and meagre at this level, the increase in tolerance thus being eight-fold (Table 3).

3. *Cross-resistance studies* :

Cheshunt compound adapted strain of *S. rolfsii* was transferred to 20,000, 50,000 and 75,000 μg. of copper supplied as copper sulphate. Similarly the strain of *S. rolfsii* resistant to copper sulphate was transferred to 1500, 2000 and 2500 μg. of copper in the form of Cheshunt compound. Surprisingly the strain resistant to copper sulphate was no more resistant than the parent strain on Cheshunt compound as evidenced by complete failure of this strain to show any growth in any of the treatments. Cheshunt compound adapted strain showed growth in all the treatments with copper sulphate (Table 4).

TABLE 4 : Growth of Cheshunt compound adapted strain of *S. rolfsii* on copper sulphate

μg. of copper as copper sulphate	Growth in mg.	Sclerotial ratings
20000	468	+++
50000	278	0
75000	128	0

DISCUSSION : A two-fold increase in tolerance to cheshunt compound (2000 μg. of copper per flask) was shown by the fungus even at the first sub-culturing. Buxton (1960) attributes this type of tolerance to wild type characteristic as it is unlikely that the fungus could adapt itself so suddenly in the first sub-culturing itself. To tolerate concentrations higher than 2000 μg. of copper in the form of Cheshunt compound, however, the fungus required a long training process and, in fact, this type of adaptation is more common among micro-organisms.

In copper sulphate experiments the fungus has not shown any marked adaptive tendency to tolerate higher concentrations upto 8th generation when it tolerated only 13,500 μg. of copper in contrast to initial tolerance of 9500 μg. It was interesting that a sudden fluctuation in its adaptive nature was evident at the eighth transfer when the fungus grew luxuriantly even in 15,500 μg. Such spontaneous and inheritable

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

changes observed both in Cheshunt compound as well as copper sulphate can be attributed to mutations. Emerson and Cushing (1946), Christensen and Daly (1951) and Roper and Kafer (1957) have suggested mutation as the mechanism governing adaptation in fungi. When the adapted strain of *S. rolfsii* was returned to fungicide—free medium, it retained its resistance during the three successive generations. It clearly suggests that the acquired tolerance was inherited even in the absence of the fungicide and thus adaptive change appears to be a permanent one, and this adds weight to the mutation theory. Partridge and Rich (1962), however, concluded that the induced tolerance to fungicides in three species of fungi studied by them probably involved adaptive enzyme formation.

When the nutrient medium was solidified with agar, the fungus tolerated much higher concentrations of copper than those in liquid medium. Parry and Wood (1958) obtained similar results with *Botrytis cinerea* grown on a medium containing copper sulphate. These results suggest that certain components of agar are antagonising the effect of copper. It was found that by incorporating 500 μg. of iron per flask in the medium, the parent strain of *S. rolfsii* could easily tolerate 3000 μg. of copper in the form of Cheshunt compound while a concentration of 1500 μg. was found toxic in the absence of iron. This result is in agreement with the eariler finding of Apparao (1959). It is very likely that agar might be containing considerable amounts of iron which are sufficient to antagonise copper toxicity. The iron content of agar was therefore analysed and it was found that 108 μg. of iron was present in the agar in each petriplate. It is quite likely that iron is responsible for tolerance of high quantities of copper by *S. rolfsii* in solid medium than in liquid medium. The effect of other components present in agar cannot, however, be ruled out, in view of the fact that adaptation in the case of Cheshunt compound is not for copper alone but to ammonia also.

The behaviour of the Cheshunt compound adapted strain in copper sulphate and *vice versa* was studied. The Cheshunt compound adapted strain could tolerate the levels of copper tolerated by the copper sulphate adapted strain while the converse was not possible. This clearly suggests that the acquired resistance of Cheshunt compound adapted strain was not to copper moiety alone but also to ammonia as the copper sulphate adapted strain could not grow in Cheshunt compound where ammonia was present in addition to copper. This indicates that adaptation to copper and ammonia is independent. It also proves the highly toxic nature of ammonia to *S. rolfsii* since the strain adapted to copper alone could not tolerate increasing amounts of Cheshunt compound, where ammonia is present.

SUMMARY

By successively transferring the mycelium in media containing progressively higher concentrations of Cheshunt compound and copper sulphate for twelve successive generations distributed over six months, a strain of *S. rolfsii* which could tolerate 3000 μg. of copper in the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

form of cheshunt compound and 80,000 μg. of copper in the form of copper sulphate in contrast to 1000 μg. and 9500 μg. of copper tolerated by the parent strain respectively was obtained. The resistance of the fungus was observed only when sclerotial initials were used as inoculum.

When the basal medium was solidified with agar the fungus tolerated much higher concentration of the toxic substance than in liquid medium.

The strain of *S. rolfsii* which was resistant to Cheshunt compound was also resistant to copper sulphate. But the strain adapted to copper sulphate was no more resistant than the parent strain to Cheshunt compound.

When the adapted strain was returned to fungicide—free medium and grown for three successive generations distributed over six weeks, it still retained its tolerance to the fungicide.

AKNOWLEDGEMENT : We are thankful to Dr. Mir Hamid Ali, Reader and Head, Department of Entomology and Plant Pathology and Dr. H. A. Razvi, Principal, for their interest and facilities provided throughout this work.

College of Agriculture,
Osmania University,
Rajendranagar, Hyderabad.

REFERENCES

APPARAO, A. (1959) Iron copper antagonism in growth of *Piricularia oryzae*. *Curr. Sci.*, **28** : 336.

BUXTON, E. W. (1960) in *Plant Pathology. An advanced treatise* **2** : 387-405. Edited by Horsfall and Dimond. Academic Press, New York and London.

CHRISTENSEN, J. J. AND J. M. DALY, (1951) Adaptation in fungi. *Annu. Rev. Microbiol.*, **5** : 57-70.

EMERSON, S. AND J. E. CUSHING, (1946) Altered sulphanamide antagonism in *Neurospora*. *Fed. Proc.*, **5** : 379.

GATTANI, M. L. (1951) Adaptation of fungi to fungicides and its significance in Agriculture. *Indian Phytopath.*, **4** : 174-180.

GROVER, R. K. AND J. D. MOORE, (1961) Adaptation of *Sclerotinia fructicola* and *Sclerotinia laxa* to higher concentrations of fungicides. *Phytopathology*, **51** : 399-401.

HORSFALL, J. G. (1956) *Principles of fungicidal action.* 280 pp. Waltham, Mass.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PARRY, K. E. AND R. K. S. WOOD, (1958) The adaptation of fungi to fungicides—Adaptation to copper and mercury salts. *Ann. Appl. Biol.*, **46** : 446-456.

PARTRIDGE, A. D. AND A. E. RICH, (1962) Induced tolerance to fungicides in three species of fungi. *Phytopathology*, **52** : 1000-1004.

ROPER, J. A. AND E. KAFER, (1957) Acriflavine-resistant mutants of *Aspergillus nidulans*. *J. gen. Microbiol.*, **17** : 69.

STAKMAN, E. C., F. V. STEVENSON, AND C. T. WILSON, (1946) Adaptation of monosporidial lines of *Ustilago zeae* to arsenic. *Phytopathology*, **36** : 411.

TAYLOR, J. (1953) The effect of continual use of certain fungicides on *Physalospora obtusa*. *Phytopathology*, **43** : 268-270.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

NEW RECORDS OF PLANT DISEASES FROM HIMACHAL PRADESH

H. S. SOHI, S. S. JAIN, S. L. SHARMA AND B. R. VERMA

(Accepted for publication December 20, 1963)

During the course of periodic surveys of Government farms and Orchards in the Territory, a number of diseases of important plants were studied, out of which seven are new records for Himachal Pradesh. Frosty mildew of apricot and *Ascochyta* leaf spot of maize are new records for India.

1. Leaf spot of ginger (*Phyllosticta zingiberi* Ramakr. T.S.)

During October, 1962, a serious leaf spot was observed on ginger at Crop Research Station, Dhaulakuan (1800′ m.s.l.) in Sirmur District, causing extensive loss to the crop by destroying chlorophyll of the leaves. The disease was characterised by the formation of oval to elongate or sometimes irregular spots with white papery centres and brown margins, 2 mm.—15 mm. in size, with extending yellowish halos, usually isolated, but sometimes confluent, covering a large part of the leaf blade, thus causing extensive discoloration and drying of the leaves. Numerous black dot-like pycnidia scattered on the white papery portion of the spots were observed, which on identification closely resembled *Phyllosticta zingiberi* Ramakr. Pycnidia were ostiolate, amphigenous, subglobose to globose, dark brown, erumpent and measured 57.3-145μ in diameter. Spores were hyaline unicellular, oval to oblong, biguttulate and measured 3.8-7.64 $\times$ 3.0-3.8μ. The present fungus differs from the one described by Ramakrishnan (1942) in the width of the spores, which was 1.2-2.5μ compared to 3-3.8μ of this fungus.

Ramakrishnan (1942) pointed out that the fungus did not cause much damage to the crop under Madras conditions, but it can assume importance if the conditions are favourable for its development. Under Dhaulakuan conditions, the attack of the disease was very severe as most of the leaves of the affected plants were involved.

2. Septoria Leaf spot of ginger (*Septoria zingiberis* Sund.)

Another leaf spot of ginger leaves was observed at Chambaghat (Solan) as well as at Dhaulakuan during the months of October and November, 1962. Spots spindle-shaped, 2-5 mm., with whitish papery centres and brown-coloured margins. Spots isolated or sometimes they coalesce to cover large portions of the leaves. Pycnidia dark coloured erumpent over the white portions of the spot, ostiolate, globose and measure, 87.86-114.6μ in diameter. Spores hyaline to very light brown in colour, 1 to 3 septate, smooth, straight or slightly curved with tapering apex, measuring 13.37-21.0 $\times$ 3.0-3.8μ. The only record of this fungus from India so far is by Sundaram (1961) from Wynaad (Madras State).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

3. Leaf spot of maize (*Ascochyta* species)

The disease was observed only on the inbred parent of Ganga 101 variety of Hybrid maize at Dhaulakuan. Spots elliptical to irregular, amphigenous, with whitish centres and brown-coloured margins, having yellowish halos and measure 1.5 to 12 mm. by 1 to 9 mm. In severe cases of infection, spots coalesce with each other and form larger diseased areas. The infection was generally more severe towards the tips of the leaves, while the mid-ribs were free from it.

Pycnidia numerous, scattered on the whitish centres, on both surfaces of the leaves, but more prominent on the upper surface of the spots, dark brown to black, sub-epidermal, globose to depressed globose and measure 95.5 – 152.8μ with a well-marked ostiole, 11.4 19.0μ in diameter. Conidia oval to oblong, sometimes cylindrical, hyaline, generally biguttulate but sometimes with 1—3 or more guttulae and measure 6.8 – 11.4 × 3 – 3.8μ, non-septate or with an indistinct septum. These dimensions do not fully agree with those of any of the described species of *Ascochyta* on maize. This appears to be the first record of this fungus on maize in India.

4. Frosty Mildew of Apricot (*Cercosporella persicae* Sacc.)

A severe leaf spot of apricot (*Prunus armeniaca* L.) was observed at Gopalpur in Mahasu District during November, 1962 on young nursery plants. The disease was characterised by the formation of circular to irregular or indefinite yellow spots on the upper surface of the leaves, varying from 2-7 mm. in size. These later turn reddish brown and ultimately become dark brown. On the lower surface of the leaves, a characteristic growth giving an appearance similar to that of powdery mildew was also observed during later stages of the disease. Affected leaves shed prematurely. The spots prior to defoliation become necrotic.

The whitish powdery mass was composed of mycelial mat and conidia. Conidia were hyaline, vermicular to clavate, having mostly 2-10 septa and measure 14.2 – 76.0 × 3.8 – 7.6 μ. This appears to be the first record of this fungus on this host in India.

5. Fruit rot of Brinjal (*Ascochyta lycopersici* Brun.)

The disease was observed on brinjal fruits at Dhaulakuan during October-November, 1962. It was usually observed on the sepals in the beginning and then attacked the adjacent portions of the fruit. The affected tissues were discoloured with depressed margins, sometimes spots showed conspicuous zonation. Numerous pycnidia were observed on the central portions of the diseased spots. Pycnidia were light to dark brown, scattered or in bands, globose to sub-globose, deeply embedded to erumpent, ostiolate, 150 – 350 μ in diameter; Ostiole distinct, 48.9 – 65.2 μ in diameter. During later stages of infection, the pycnidia become gregarious and in some cases may coalesce with one another. Spores hyaline, ovoid to oblong, rarely uni- but mostly bi-cellular, with a clear constriction at the septum, 4.6 – 11.6 × 2.3 – 3.5μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The pycnidial and spore measurements of the present specimen do not agree with those of any species of *Ascochyta*, reported from India on brinjal. Padmanabhan (1947) has reported *A. melongenae* on brinjal seedlings only.

6. Wilt of Black gram (*Fusarium* species).

At the Crop and Vegetable Research Station, Solan, a severe wilting of black gram (*Phaseolus mungo* L.) was observed in a varietal trial during mid-September, 1962. In initial stages, lower leaves of the plants showed a gradual change of colour from green to yellow. With the advance of the disease, upper leaves also showed discolouration and withering, resulting in severe shedding ultimately. The root system was found to be normal in initial stages, while in advanced stages of the disease, rotting and discolouration of secondary roots was also observed. Isolations from the diseased roots have yielded a species of *Fursarium*. Both micro and macroconidia were formed on Oat meal Agar.

7. Bacterial Leaf spot of Pomegranate (*Xanthomonas punicae* Hingorani and Singh,

During October, 1962, a leaf spot of pomegranate (*Punica granatum* L.) was observed on many plants in Solan area. The infection was generally confined to the leaves but in a few cases the fruits were also attacked. The spots were circular to irregular in shape, varying from less than a mm. to more than 5 mm. in diameter and light to dark brown in colour. In cases of severe infection, adjacent spots may coalesce with each other to cover large areas and the leaves turn yellow. Microscopic examination of diseased lesions revealed the presence of a large number of bacteria, which resembled *Xanthomonas punicae* described by Hingorani *et al*, (1960) from Delhi.

ACKNOWLEDGEMENTS : Grateful thanks are due to Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh, for his keen interest in this work and encouragement.

Section of Plant Pathology, Department of Agriculture,
Himachal Pradesh, Solan.

REFERENCES

HIGGINS, B. B. AND A. Wolf, (1937) Frosty mildew of peach. *Phytopathology*, **27** : 690.

HINGORANI, M. K. AND N. J. Singh (1960) *X. punicae* sp. nov. on *Punica granatum L. Indian J. Agric. Sci.*, **29** : 45-48.

*NICOLAS, G. AND Mile. Aggery, (1928) La fonte des jeunes plants d' Aubergine (The damping-off of young egg plants). *Rev. Path. Veg. et. Ent. Agric.* **15** : 6, 149-151. Abs. in *Rev. appl. Mycol.* **8** : 10, 1929.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PADMANABHAN, S. Y. (1947) A new seedling disease of Brinjals. *Indian J. Agric. Sci.*, **17** : 393-395.

*RAMAKRISHNAN, T. S. (1942) A leaf spot disease of *Zingiber officinale* caused by *Phyllosticta zingiberi* n. sp. *Proc. Indian Acad. Sci.*, Sect. B, **15** : 4.
Abs. in *Rev. appl. Mycol.*, **21** : 471, 1942.

SINGH, U. B. (1943) Some diseases of fruits and fruit trees in Kumaon-I. *I.C.A.R., Misc. Bull.* **51** : 16.

STEVENS, F. L. AND ATIENZA J. D. (1931) : Diseases of cultivated ginger. *Phillpp. Agric.* **20** : 3, 171-176. Abs. in *Rev. appl. Mycol.*, **11** : 73-74. 1932.

SUNDARAM, N. V. (1961) : Notes on some fungi from South India. *Indian Phytopath.*, **14** : 202-209.

---

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FURROW APPLICATION OF FUNGICIDES FOR CONTROL OF COTTON SEEDLING DAMPING-OFF IN ISRAEL*

Z. SOLEL AND G. MINZ

(Accepted for publication December 30, 1963)

Cotton seedling damping-off is known to occur in most cotton-growing areas of Israel (Solel and Palti, 1960). The fungi causing the disease are *Rhizoctonia solani* Kühn and *Fusarium* spp.; occasionally *Sclerotium bataticola* Taub. was also isolated from diseased seedlings. Reports from the U.S.A. (Bird *et al.*, 1957; Ranny and Bird, 1956) show good results in controlling this disease by mixing fungicides with soil covering the seeds of cotton.

The purpose of the experiments carried out during the years 1960-1962 was to determine the efficacy of several fungicides in controlling cotton damping-off, and the effectiveness of different methods of mixing fungicides with the soil under local conditions (fungi, soil and climate).

MATERIAL AND METHODS: All the experiments were designed in randomized blocks with six replications. Plots were 20-25 meters long, and consisted of four rows of 1 m. width each.

Seeding was done during April, to a depth of 3-4 cm. To enable good germination the soil was irrigated several times after seeding. The experiments were conducted on several farms, with different soil types, crop rotations, and climates.

The following fungicides were tested:

1.	Brassicol 75 W. P.[a]	[Pentachloronitrobenzene (PCNB) 75 per cent]
2.	Brassicol 60[a]	(PCNB 60 per cent)
3.	Terraclor 75 W. P.[b]	(PCNB 75 per cent)
4.	Micid 55 W. P.[c]	(Zineb 65 per cent)
5.	Orthocide 50 W. P.[d]	(Captan 50 per cent)

*Contribution from The National and University Institute of Agriculture, Rehovot, Israel. 1963 Series, No. 582-E.

[a]produced by Farbwerk Hoechst AG., Germany.

[b]Produced by Olin Mathieson Chemical Co., U.S.A.

[c]Produced by Abic Ltd., Israel.

[d]Produced by California Spray Chemicals Co., U.S.A.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Two methods of application were tested :

1. "In-the-furrow" method, described by Ranney and Hillis (1958), two cone nozzles are used. One nozzle was placed immediately behind the seed spout, which sprayed the seedbed and the soil in the furrow, while the other was adjusted in a slightly posterior position, thus spraying on to the soil as it filled the furrow and covered the seeds. The amount of spray used was 20.1 per dunam*.

2. The planter-box method ("In-the-hopperbox"), was used for dusting. The amount of fungicide used was determined according to the quantity of seeds sown per dunam (2-4 kg. seed/dunam). The dust flowed through the seed spout, and was mixed with the soil surrounding the seed.

Infection rates were calculated as percentage of dead seedlings (counted every few days) from plants one month after emergence. Dried-up seedlings were collected for culturing.

RESULTS : The results of these experiments are summarized in Table 1. The stand was low in 1960 at Beit Dagan, apparently because of adverse growing conditions (extremely high temperatures and low humidity) during the fortnight after sowing. It is possible that the low percentage of damping-off was due to these environmental conditions. Nevertheless, the effect of the treatments, and the difference between fungicides could be clearly observed. Both Terraclor and Micid significantly reduced (P=0.05) the post-emergence damping-off, the first fungicide being somewhat superior to the second. Orthocide, on the other hand, significantly increased the incidence of the disease, as compared to control plots.

During the second year (1961) at Biet Dagan, the disease was more conspicuous, but the results were basically the same as those of the previous year. In this year we added examination of the effect of the treatments on stand. Counts of plants which emerged showed significant (P=0.05) promotion of emergence by Terraclor. The other fungicides did not show any significant positive effect, but Orthocide had a negative effect on emergence.

In 1962 experiments were carried out at three other localities, representing different soil types, crop rotations and climates. During this year with planter-box method of fungicide application, differences in emergence were very slight, and it can be concluded that the chemicals had no effect on emergence. Terraclor and Brassicol (both products of PCNB) were highly significant in reducing the incidence of the disease at Sa ad and Miriam farm (east of Ashquelon). In Sa ad : the fungicidel results were interesting reducing the infectihn from 16.2 % to 1.4%. Micid was also positive, but less efficient than the PCNB products. The planter-box method proved as efficient as in-the-furrow method.

DISCUSSION : Different authors have reported contradictory conclusions derived from data obtained through bioassays concerning

*1dunam=1000 m²=approx. 1/4 acre

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : The effect of fungicides applied to soil by "in-the-furrow" method (spray), and by "planter-box method" (dust), on emergence and on infection rate of cotton seedlings by fungi

Locality	Beit Dagan				Na'an			Miriam Farm		
Year	1960		1961		1962			1962		
Material	Rate of fungicide (g/du)	Infection rate of seedling (%)	Seedling emergence (no.)	Seedling infection (%)	Rate of fungicide (g/du)	Seedling emergence (no.)	Seedling infection (%)	Rate of fungicide (g/du)	Seedling emergence (no.)	Seedling infection (%)
Terraclor (spray)	400	0.8	10.656	0.8	450	8,550	1.2	450	5,180	0.4
Brassicol (spray)	—	—	—	—	450	8,080	1.2	450	15.960	0.1
Brassicol (dust)	—	—	—	—	—	—	—	500	15,580	0.1
Micid (spray)	500	1.1	9,452	2.6	450	8,590	0.8	450	16,530	1.2
Orthocide (spray)	600	4.9	8,662	14.3	—	—	—	—	—	—
Control	—	2.4	9,286	9.0	—	8,580	1.3	—	14,880	5.2

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the specific toxicity of fungicides to various fungi.

Bird *et al.*, (1957) and Domsch (1959) had shown that PCNB was highly toxic to *Rhizoctonia solani*, whereas Vaartaja (1960) indicated that PCNB was a relatively week toxicant to that fungus. Vaartaja found a toxic effect of Zineb on *R. solani* and *Fusarium solani*, while Domsch considered Zineb to be a very weak toxicant to *F. culmorum* and to *R. solani*. The effect of Captan is also under dispute ; both Vaartaja and Domsch found it to be ineffective in suppressing *Fusarium* spp., but the former showed it to be toxic to *R. solani*, whereas the latter found only a very slight effect of Captan on that fungus. Sinclair (1960) however found various degrees of susceptibility of five isolates of *R. solani* to several fungicides.

In our field tests, the most prevalent fungus isolated from diseased seedlings was *R. solani* ; in only a few cases *Fusarium* sp. was isolated. This may explain the positive effect of PCNB and Zineb. The negative effect of Captan was unexpected because there are recommendations for using it as a control measure for damping-off (Bird *et al.*, 1957 ; Ranny and Bird, 1956). But there may be some assumptions for explaining this phenomenon. Ranney and Bird (1956), while testing several fungicides, including Captan, concluded that "There is the possibility that at 80° F the fungicides applied reduces the normal soil-born organisms antagonistic to *Fusarium* spp. thus permitting increased activity of the pathogens." The same view has also been expressed by Bird *et al.*, (1957) ; support for this mechanism may be found in the results given by Domsch (1959).

At Beit Dagan in 1961 there was a positive effect of Terraclor on the emergence rate. This was probably due to reduction in pre-emergence damping-off, which did not occur in any other tests. Unfortunately the incidence of the disease in all experiments was low, and therefore, a pronounced effect of the treatments on yields could not be expected. Taking into consideration that the usual practice in Israel is to sow a dense stand with subsequent thinning out of the plots, a relatively small percentage of dried-up seedlings might not affect the yield.

From the practical point of view of the farmer, it is worthwhile noting that the planter-box method was easier to use than the "in-the-furrow" method, and was more satisfactory in results.

SUMMARY

Five chemicals were tested for controlling damping-off, mainly caused by *Rhizoctonia solani*. Good results in reducing the percentage of infection (post-emergence) were attained with PCNB and Zineb, the former being superior to the latter. Captan caused an increase in the disease rate. Both methods of application, spraying the fungicides into

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the furrow, or dusting by mixing the fungicides with the seed in the planter-box gave good results.

Division of Plant Pathology,
The National and University Institute of Agriculture,
Rehovot, Israel

References

BIRD, L. S., C. D. RANNEY AND G. M. WATKINS. (1957) Evaluation of fungicides mixed with the covering soil at planting as a control measure for the cotton seedling disease complex. *Plant Dis. Reptr.* **41** : 165-173.

DOMSCH, K. H. (1959) Untersuchungen zur Wirkung einiger Bodenfungiziden. 32 Deutsche Pflanzenschutz-Tagung : pp. 100-106.

DOMSCH, K. H. (1959) Die Wirkung von Bodenfungiziden. III. Quantitative Veränderung der Bodenflora. *Z. Pflkrankh.* 66 : 17-26. (*Rev. appl. Mycol.*, **38** : 455,1959).

RANNEY' C. D. AND L. S. BIRD (1956) Greenhouse evaluation of in-the-furrow fungicides at two temperatures as a control measure for cotton seedling necrosis. *Plant Dis. Reptr.*, **40** : 1032-1039.

RANNEY, C. D. AND A. M. HILLIS. (1958) Method of Applying Fungicides in the Covering Soil at Planting for Controlling Seedling Disease of Cotton. *Rep. Texas agric. Exp. Sta.* No. 2001.

SINCLAIR, J. B. (1960) Reaction of *Rhizoctonia solani* isolates to certain chemicals. *Plant Dis. Reptr.*, **44** : 474-477.

SOLEL, Z. AND J. PALTI. (1960) Cotton seed rot and damping-off. *Hassadeh*, **40** : 685-686 (Hebrew). (*Rev. appl. Mycol.*, **40** : 468, 1961).

VAARTAJA, O. (1960) Selectivity of fungicidal materials in agar cultures. *Phytopathology*, **50** : 870-873.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FUSARIUM SEEDLING BLIGHT OF MAIZE IN RAJASTHAN

H. C. ARYA AND B. L. JAIN

(Accepted for publication December 20, 1963)

INTRODUCTION : Maize (*Zea mays* L.) is an important rainy season crop in Rajasthan. The total area under cultivation is nearly one million acres with an average yield of 0.1 million tons. In August 1957, an epiphytotic of seedling blight was observed in fields near Mount Abu. *Fusarium* was isolated from infected seedlings collected from these fields.

Pammel (1914) investigeted a serious root and stalk disease of maize, caused by *Fusarinm* in Iowa, U.S.A. Hoffer *et al.*, (1918) investigated the importance of *Gibberella saubinetti* (Mont.) Sacc. in the root rot syndrome of maize. Numerous isolations of *Fusarium moniliforme* (sheld.) Wine. from rotting roots and stems of maize seedlings were made by Valleau (1920) in Kentucky. He was inclined to believe that *F. moniliforme* was the primary cause of root rot and stalk rot of maize in that State and also in the other central and southern States. Both pre-and post-emergence seedling blights have been studied in detail and the occurrence of low temperature has been positively correlated with disease development (Dickson, 1923; Dufrenoy and Fremont, 1931). Dickson (1923) recorded 3 species of *Fusarium* responsible for the disease : *F. moniliforme* var. *subglutinans* Wr. and Reinking, *F. moniliforme* and *F. graminearum* Schw.

F. moniliforme was found to be a root rotting pathogen of maize under field conditions by Holbert *et al.* (1925). Valleau (1926) concluded from their study on maize root rot that *F. moniliforme* was a semi-parasitic organism that destroyed temporarily the root system. Pearson (1931) worked out the details of host parasite relations in the corn seedling blight caused by *Gibberella zeae* (Schw.) Petch, an ascigerous stage of *F. graminearum*. Leonian (1932) investigated the pathogenicity and variability of *F. moniliforme* and found that it was very unstable. According to Wen Chun Ho (1944), this fungus possessed only a slight virulence and was chiefly saprophytic. Niederhauser (1950) described it as the principal rotting agent of roots, stems and ears of maize. Podhradszky (1950) reported heavy losses of maize due to attack by *F. moniliforme*.

There are no previous reports of this disease from India. It was, therefore, considered important that a study be made on taxonomy, physiology and pathogenicity of the fungus. The results are presented in this paper.

MATERIAL AND METHODS : The isolates used in these tests were obtained from infected seedlings raised in the field. An authentic culture of *F. moniliforme* was procured from type culture collection

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

at the Indian Agricultural Research Institute, New Delhi, for comparative studies. The isolate has been called in the text as *Fusarium* isolate.

Unless otherwise specified, the local variety of maize grown in Jaipur, Rajasthan was used in all pathogenicity tests. Three other varieties of maize, Udaipur-selected, Basi-selected and Malan-white were obtained from the Department of Agriculture, Rajasthan, for testing their reaction to the disease. Tests concerning pathogenicity were conducted under green house conditions on seedlings grown in clay pots. The fungus was grown on corn meal sand medium as suggested by Rawlins (1933). Both solid and liquid media were used in cultural and physiological studies with 6 to 12 replicates per test and each test was repeated three times.

EXPERIMENTAL RESULTS : *Symptoms and effects* : The fungus in the pre-emergence stage invaded the micropylar region of the germinating maize seeds and killed the emerging radicle and the plumule. Mycelium grew extensively and rotted the host tissue. The emerged radicle and plumule bore elongated reddish-brown lesions. The hypocotyl region proved susceptible to attack and characteristically developed brown spots. After emergence the seedlings grew to the three leaf stage of development. Then leaf chlorosis began at the apices and progressed toward the base followed by severe wilt and ultimately death of some plants. Plants often recovered from preliminary infection but mortality ranged from 60 to 70 per cent.

Heavy infection of the cortical cells in the hypocotyl and radicle was observed which produced plasmolysis and browning of the cell walls. The mycelium was both inter—and intra-cellular. Infection of vascular tissues also occurred as evidenced by the presence of mycelium in the xylem vessels (fig. 1A). The fungus entered the host tissue either through root hairs or the epidermis, where it formed extramatrical fungal mass. Formation of appresorium has also been noted on the point of entrance in the root hairs. (fig. 1B).

Morphology of the fungus : The colony colour of the fungus on potato-dextrose-agar (P. D. A.) was cottony-white during early growth, but later it changed to light violet. A comparative study of the morphological characters of *F. moniliforme* and the *Fusarium* isolate did not reveal any important differences other than colony colour.

The microconidia were produced either singly or in chains or in pseudo-heads over the conidiophores (fig. 1 C & D). The microcondia were 0-2-septate and spindle or ellipsoidal in shape. The chains usually consisted of 5-7 conidia. They were produced abundantly in the culture and measured $4.5-11.0 \times 1.5-5.0\ \mu$, averaging $7.5 \times 3.0\ \mu$. Macroconidia were sickle-shaped with a foot-like base, mostly 3-5 septate and were sparingly formed. They measured $25.0-30.0 \times 7.0-8.0\ \mu$, averaging $28.0 \times 7.5\ \mu$ (fig. 1 E). Chlamydospores were formed abundantly in terminal or intercalary position on the hyphae. They

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

were globular in shape and measured 4.5 – 14.0 × 4.5 – 12.5 μ, averaging 7.0 × 6.0 μ (fig. 1 F).

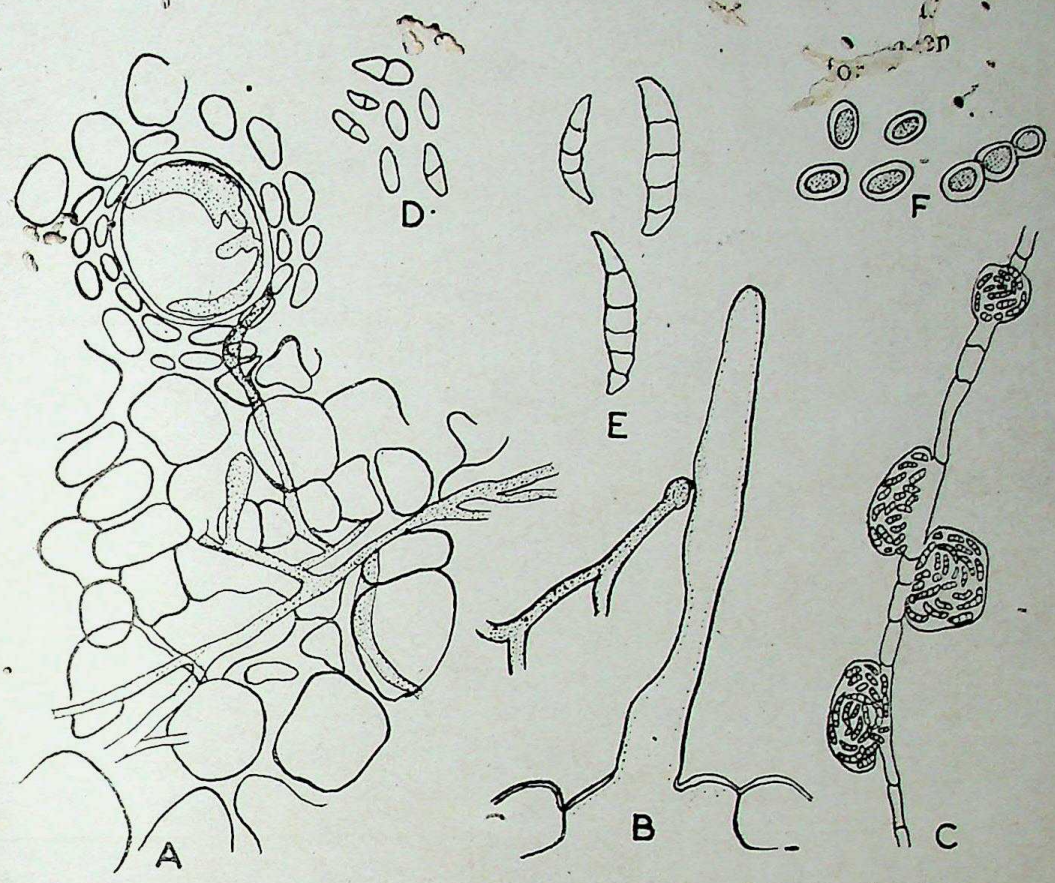


Fig. 1. A. A portion of the section of the hypocótyl, showing inter-, and intra-cellular mycelium in the cortex and also in the xylem vessel. B. Formation of the appresorium on the point of entry of the fungus into root hair. C. Pseudopionnotes. D. Microconidia. E. Macroconidia. F. Chlamydospores. Magnification, ×425.

Cultural characters of the fungus : On PDA medium the isolate formed a white colony of about 19 mm. diameter after three days' growth at a pH of 6.5 and a temperature of 27°C. After nine days the colony reached a diameter of nearly 85 mm. The colony colour later became light pinkish-violet. The fungus was found to grow well on a number of different nutritive media although best growth occurred on Richard's, Corn-meal, and Czapek agar. It had comparatively much faster growth rate than *F. moniliforme* on PDA. The colony colour of *Fusarium* isolate on rice media (Maerz and Paul, 1930) was reddish-pink, compared to the white colour of *F. moniliforme* on this medium as well as on PDA.

The fungus grew well on PDA between 18-32°C with an optimum at about 26°C. At 36°C no growth occurred but if the culture was placed at 26°C within 72 hours, growth resumed. Death of the fungus occurred if placed at 41°C for 3 hours. The fungus grew over a pH range from 3.5 to 9.5 with an optimum at 5.5.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Effect of Temperature on pathogenicity : The seed was inoculated by dipping in a mycelial suspension and also inoculating soil at monthly intervals beginning in July and ending in December, 1958 in the green house. During this period, the temperature ranged from 18°C to 37°C (Table 1). The sterilized soil was placed in earthern pots which had been previously steam sterilized. The soil was inoculated one week prior to sowing the seed to allow for colonization of the soil. The control consisted of non-inoculated seed and soil. Both pre-and post-emergent blight was favoured by low temperatures which were prevalent during the period September to December (Table 1). However, pre-emergent blight occurred even under prevailing high temperatures. Post-emergent blight was not observed until the cooler temperatures of November and December prevailed. No infection was recorded in the control experiments. These results were similar to those recorded by Dickson (1923) who stated that the cases of severe blight were obtained at about 20°C.

TABLE 1 : Effect of temperature on seedling blight (Soil moisture 50-75 per cent)

Period of inoculation 1958	Temperature in°C		No. of seeds sown	Pre-emergence blight	Post-emergence blight
	Max.	Min.			
July	37	27	100	45	0*
August	33	27	100	45	0*
September	32	27	100	50	0*
October	28	22	100	55	0*
November	26	20	100	65	10
December	24	18	100	70	15

*At the post-emergence stage only the first leaf showed browning.

Effect of Moisture on pathogenicity : Zinc-plated iron pots were used. The soil moisture was maintained at approximately 75, 50, 25, and 10 per cent levels. The results obtained showed that the maximum seedling blight took place at 75 per cent soil moisture, while it was least at 10 per cent soil moisture. At 10 to 25 per cent moisture level, the plant growth itself was considerably reduced.

A comparative study of the pathogenicity of *F. moniliforme* and *Fusarium* isolate revealed that the latter caused considerably more seedling blight on the local variety of maize under optimum temperature and soil moisture.

Varietal reaction : Reaction of 4 maize varieties (Local, Basi-selected, Udaipur-selected, and Malan-white) was studied under green

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

house conditions. The varieties were sown separately in 3 types of soils, namely calcareous, sandy, and well-manured. Each soil type was divided into two lots, sterilized, and unsterilized. Control experiments with uninoculated seeds of each variety were also set up for comparative study. The results are presented in Table 2.

TABLE 2 : Per cent blight of maize varieties to infection by *Fusarium* isolate. (Temperature 20-26°C. ; Soil moisture 50-75 per cent)

*Maize variety	No. of inoculated seeds sown	Pre-emergent blight & Post-emergent blight					
		Unsterilized soil			Sterilized soil		
		Calcareous	Sandy	Manured	Calcareous	Sandy	Manured
Local	100	75	50	30	55	40	20
Basi-selected	100	75	60	60	65	60	50
Udaipur-selected	100	15	15	0	10	10	0
Malan-white	100	20	15	10	20	10	5

*In the control experiments no infection was recorded.

Local, and Basi-selected maize varieties proved highly susceptible. Udaipur-selected, and Malan-white varieties were found to be resistant. The results in sterilized and unsterilized soils did not show major differences although more infection was obtained in sterilized soil in 9 out of 12 comparisons. More blight was obtained in calcareous soil and least in manured soil.

DISCUSSION : Three species or varieties of *Fusarium* have been previously reported to be causing seedling blight of maize (Dickson, 1923). They are *F. moniliforme*, *F. graminearum*, and *F. moniliforme* var. *subglutinans*. The present isolate, differs, however, in the following characters : 1. Microconidia were produced abundantly either in chains or pseudopionnotes. 2. Macroconidia were produced only sparingly. 3. Chlamydospores were produced in abundance.

The *Fusarium* isolate also differed in cultural and physiological characters from *F. moniliforme* obtained from the type culture collection at the I. A. R. I., New Delhi. Leonian (1932) stated that most of the strains of *F. moniliforme* were very plastic and dissociated into many forms. Even on monosporic

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

isolations, it produced no less than 50 variants differing among themselves not only in pathogenicity, but also in morphological and physiological characters. Recently Venkata Ram (1957) has also coroborated the above statement in his work on the soil Fusaria and their pathogenicity.

The fungus under study appears to be a virulent strain of *F. moniliforme*.

The fungus was more active to cause disease under relatively low temperatures (18-26°C.) and high moisture conditions. This may be the reason why the disease was confined to the mountainous area where suitable environmental conditions prevail during crop season.

Out of four varieties of maize which were tested for the varietal reaction to the disease, two showed remarkable resistance. Further investigation in this direction will be useful to devise suitable measures for the control of the disease.

SUMMARY

A fungus has been recorded causing severe seedling blight of maize in the Mt. Abu area of Rajasthan in 1957. The symptoms of the disease have been described.

The characteristics of the disease and morphology of the fungus had close resemblance with *Fusarium moniliforme* However, certain differences in colour, growth rate, and fructifications were found. The present isolate was comparatively more aggressive in virulence than the type culture of *F. moniliforme* obtained from I.A.R.I. Green house tests showed that the disease was severe both at the pre-emergence and post-emergence stages in the temperature range of 18-26°C. Similarly, high moisture of the soil and its calcareous nature aggravated seedling blight. Of 4 varieties of maize tested, Malan-white, and Udaipur-selected proved resistant, whereas Local and Basi-selected were highly susceptible. The fungus has been identified as a virulent form of *F. moniliforme*.

ACKNOWLEDGEMENT : The authors wish to express their sincere thanks to Dr. K. M. Gupta, the then Professor and Head of the Department of Botany, at the Jaswant College, Jodhpur for his close interest in the work. Thanks are also due to the Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for sending us the authentic culture of *F. moniliforme* for comparative studies.

Department of Botany,
University of Rajasthan,
Jaipur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

DICKSON, J. G. (1923) Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinettti*. *J. Agric. Res.*, **23** : 837-870.

DUFRENOY J., AND T. FREMONT, (1931). Influenced de la temperature Sur les reactions du mais a l' infection *Fusarienne*. *Phytopath. Z.*, **4** : 37-41.

HOFFER G. N., A. G. JOHNSON, AND D. ATANASCOFF. (1918) Corn root rot and wheat scab. *J. agric. Res.*, **14** : 611-612.

HOLBERT. J. R., W. L. BURLISON, B. KOEHLER, C. M. WOODWORTH, AND G. H. DWNGEN, (1925) Corn root, stalk and ear rot disease and their control through seed selection and breeding. *Illinois Agric. Exper. Stat. Bull.*, **255**.

LEONIAN, L. H. (1932) The pathogenicity and variability of *F. moniliforme* from corn. *W. Virginia Agric. Exper. Stat. Bull.*, **248**.

MAERZ, A., AND M. R. PAUL, (1930) *A dictionary of color*, McGraw Hill Book Company, New York.

NIEDERHAUSER, J. S. (1950) Enfermedades del maiz en Mexico Y variedades resistentes. *Foll. Misc. Ofic. Estud. esp. Mex.*, **3** : 232-235.

PAMMEL, L. H. (1914) Serious root and stalk disease of corn. *Iowa Agriculturist*, **15** : 156-158.

PEARSON, N. L. (1931) Parasitism of *G. saubinetti* on corn seedling. *J. agri. Res.*, **43** : 569-596.

POPHRADSZKY, J. (1956) Die Krankheiten des Maises in ungarn und ihre Bekampfung. *Act. agron. Acad. Sci. Hung.* **6** : 143-147.

RAWLINS, T. E. (1933) *Phytopathological and Botanical Research Methods*. John Willey & Sons. Inc. New York. 147 pp.

VALLEAU, W. O. (1920) Seed corn infection with *F. moniliforme* and its relation to the root and stalk rots. *Kentucky Agric. Exper. Stat. Bull.*, 226.

———— (1926) Corn root rot, a soil borne disease. *J. agric. Res.*, **33** : 453-476.

VENKATA RAM, C.S. (1955) Soil fusaria and their pathogenicity. *Proc. Indian Acad. Sci.*, Sect. B, **42** : 129-144.

WEN CHUN HO. (1944) Soil inhabiting fungi attacking the roots of maize. *Agric. Exper. Stat. Iowa State College, Res. Bull.*, 233.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FURTHER STUDIES ON FOLIAR NECROSIS OF POTATO

B. GANGULY* AND S. P. RAYCHAUDHURI

(Accepted for publication March 10, 1964)

A disease of potato (*Solanum tuberosum* L.) variety *Phulwa* characterised by foliar necrosis was observed in the fields of Indian Agricultural Research Institute, New Delhi. Nearly 50 to 60 per cent of the plants were found to be affected. Bawden[1] described similar disease causing foliar necrosis of potato and designated the causal virus as potato virus D. Vasudeva and Gupta[2] reported the occurrence of *Solanum* virus 6 on *Phulwa* potatoes from India and studied its reactions on differential hosts. Further studies were undertaken to determine the host range and properties as well as the effect of various chemicals and hydrogen-ion concentrations on the infectivity of the causal virus and the results are reported herein.

MATERIAL AND METHODS : Inoculation tests were carried out on healthy *Phulwa* potato plants grown in insect-proof glasshouse. Since only a few disease-free tubers of *Phulwa* potatoes could be obtained, a large number of *Nicotiana glutinosa* plants to which the virus is readily transmissible, were inoculated for multiplying the inoculum. A limited number of disease-free potato tubers of different varieties as well as other host species belonging to several families were raised in the glasshouse. For all the experiments only young vigorously growing plants of the same age were inoculated.

The inoculum was obtained by crushing the diseased leaves to fine pulp in sterilised pestle and mortar. To every gram of diseased leaf material 1 cc of water was added. The extract was then strained through muslin. The standard extract thus obtained was used in all inoculation experiments carried out, unless otherwise mentioned. Carborundum was used as an abrassive.

EXPERIMENTAL : *Transmission of the disease :* The disease was readily transmitted mechanically and the period of the first appearance of the symtoms varied appreciably during winter and summer months. In winter the symptoms generally appeared within 8-10 days and systemic spread of the disease was observed after 16-20 days. In summer, however, the symptoms were produced rather late, and these eventually got masked.

Host range of the virus : The virus was transmitted to the following solanaceous hosts :—*Solanum tuberosum* var. *Phulwa*, President and Uptodate, *Solanum nodiflorum* Jack., *Nicotiana tabacum* L., *Datura stramonium* L., *Lycopersicon esculantum* Mill. var. Sutton's Early Market and *Capsicum annuum* L. The percentage of infection varied from 82 to 100. The virus was also transmitted by grafting to *Solanum tuberosum* var.

*Present address—Plant Virus, Sub-station, Kalimpong, Distt. Darjeeling.

[1]Bawden, F. C. (1934), *Proc. Roy soc. Ser. B.*, **116** : 375-395.

[2]Vasudeva, R. S. and D. Gupta (1946). *Indian J. agri. Sci.*, **16** : 469-470.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Epicure, Arran victory, Craig's Defiance and Majestic. However, the disease could not be transmitted to *Solanum melongena* L. and *Solanum nigrum* L.

Symptoms on differential hosts :

Solanum tuberosum var. *Phulwa* : Under glasshouse conditions very mild mottling without any distortion of the leaf is produced followed by faint chlorosis. After 8-10 days necrotic spots are seen on the affected leaves. These necrotic spots finally coalesce and form larger necrotic areas. The smallest leaves soon after emergence are killed due to the appearance of the necrotic spots leaving behind diseased tissues in the form of brownish scaly structures.

Solanum tuberosum var. Uptodate, Craig's Defiance and Majestic : The first symptoms appear as necrotic spots. Veinal necrosis consisting of dark brown or black stripes is followed by top necrosis resulting eventually in death of the plant (Fig. 1).

Solanum tuberosum var. Epicure : The necrotic spots which develop as the first visible symptoms about 15-18 days after inoculation, spread rapidly and coalesce, finally killing the growing point. Top necrosis is the characteristic symptom in this case.

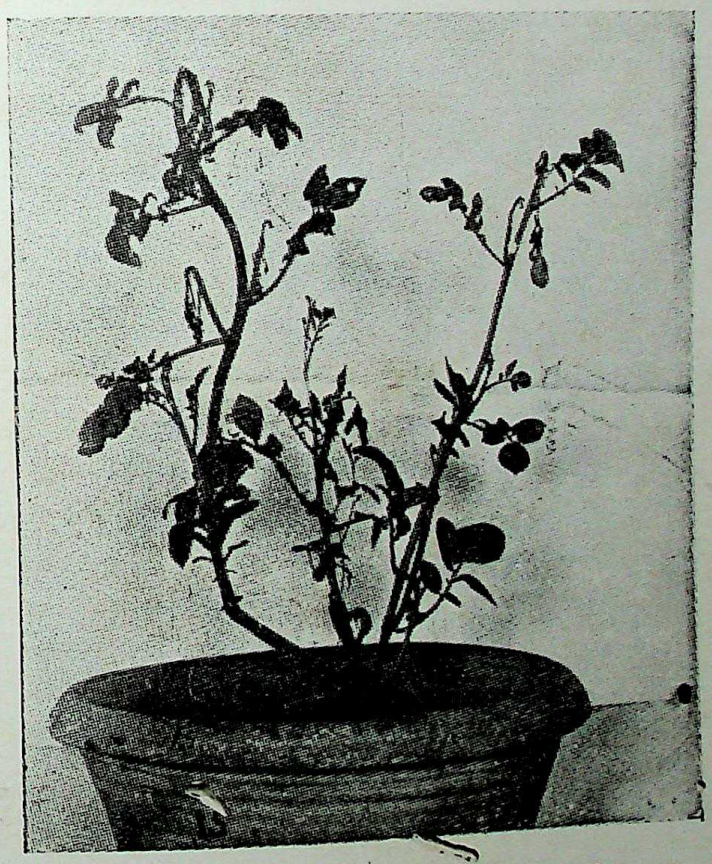


Fig. 1. Symptoms produced on *Solanum tuberosum* var. Uptodate due to infection with *Solanum* virus 6.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Solanum tuberosum var. President and Arran Victory : Interveinal necrotic spots appear 15-17 days after inoculation which spread rapidly, coalesce and ultimately the leaves wither and fall off. The disease is systemic and the growth of the plant is arrested.

Nicotiana tabacum var. Harrison's special : Pale green spots appear on leaves 12-15 days after inoculation followed by mottling and chlorosis which assume ring-like form. The disease becomes severe and the symptoms tend to disappear with advance in age 3-4 weeks after infection.

Nicotiana glutinosa : The symptoms are similar to that of *Nicotiana tabacum* var. Harrison's special excepting that the chlorotic areas frequently coalesce and form larger chlorotic areas. The chlorosis starts from the apex and spreads towards the base of the leaf.

Datura stramonium : The visible symptoms appear 10-12 days after inoculation in the young plants. The infected plants show mild mottling followed by faint chlorotic spots on leaves. After about two weeks the symptoms begin to fade and the plants carry the virus symptomlessly.

Lycopersicon esculentum var. Sutton's Early Market : The first symptoms appear 12-15 days after inoculation when infected leaves show dark and light green mottling. The tips and the margins of the leaves roll upwards and the leaves are reduced in size.

Solanum nodiflorum : The first visible symptoms are observed 12-15 days after inoculation which are almost similar to those on *Lycopersicon esculentum* var. Sutton's Early Market. Vein-clearing is followed by dark and light green mottling of the leaves. Chlorosis is observed all over the leaf surface, which later spreads acropetally.

Capsicum annuum : The first symptoms are observed 10-12 days after inoculation when mottling all over leaf surface, especially around the veins, is observed. The disease never appears in a severe form.

Properties of the virus : The dilution-end point of the virus lies between 1 : 5,000 and 1 : 10,000. The virus withstands heating upto 66°C. for 10 minutes but gets innocuous when heated to 68°C. for the same period. The longevity *in vitro* of the virus is 7 to 8 days at 25-30°C., 19 to 21 days at 4-7°C. and 39-42 days at 0-2°C.

The infectivity of the virus remains unaltered even after passing through L_2, L_3 and L_5 Chamberland candles.

For studying the reaction to various chemicals, undiluted extract of the virus and the chemical at the desired strength were mixed in equal proportions. The virus retains its infectivity when mixed with 6 per cent alcohol, equal proportions of ether and chloroform, 0.1 per cent mercuric chloride, 0.25 per cent acetone, 0.1 per cent hydrogen peroxide, 0.05 per cent potassium permanganate, and 2 per cent formalin

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and phenol. However it loses its infectivity when mixed with 70 per cent or higher concentrations of alcohol, equal proportions of glycerine, 0.25 per cent solution of mercuric chloride, 0.5 per cent hydrogen peroxide, 0.1 per cent potassium permanganate and 3 per cent formalin and phenol.

The technique employed in order to determine the effect of hydrogen-ion concentration on the virus, was the same as that described by Samuel, Best and Bald[3]. The standard extract used in these experiments was at first allowed to stand for about 24 hours at 4°C. and then centrifuged at 3,500 r.p.m. for 20 minutes in a "WIFUG" type angle centrifuge. A stock composite buffer solution of 0.0533 M with respect to each of boric acid, potassium dihydrogen phosphate and potassium hydrogen phthalate was used throughout the experiment. Aliquots of the buffer were adjusted between pH 1.0 and 11.0, at intervals of one pH unit by the addition of appropriate quantity of 0.2 M hydrochloric acid and 0.2M sodium hydroxide and distilled water to give a final solution of the desired pH value. Glass electrode was used for the determination of hydrogen-ion concentration.

The pH of the inoculum was found to be 6.8 to 7.0 when it was ready for mixing with the differently adjusted buffer solutions. One ml. portions of the centrifuged virus extract were added to 3 ml. portions of differently adjusted buffer solutions, vigorously shaken and immediately inoculated to test the effect of hydrogen-ion concentration on the infectivity of the inoculum. The data indicated that the virus remains infective between the range of pH 2.9 and 7.4.

SUMMARY

A serious disease of potato variety *Phulwa* caused by *Solanum* virus 6 or potato virus D was observed at New Delhi. Symptoms on certain hosts have been described. Among the potato varieties tested, it produces foliar necrosis on the varieties *Phulwa*, President and Arran Victory, while top necrosis is developed on varieties Up-to-date, Epicure, Craig's Defiance and Majestic.

The dilution-end point of the virus lies between 1 : 5,000 and 1 : 10,000 ; it can withstand exposure to 66°C. for 10 minutes but is rendered completely innocuous, when exposed to 68°C. for the same period. At the temperature of 25-30°C. the virus is completely inactivated during the period of 7 to 8 days ; when stored at 4-7°C., it loses its infectivity after 19 to 21 days ; at 0-2°C., it remains infectious even after 30 days but is rendered completely innocuous after storage for 39-42 days.

The virus retains its infectivity after passage through Chamberland candles L_2, L_3 and L_5.

Reactions of the virus to various chemicals, such as, alcohol, glycerine, ether, chloroform, mercuric chloride, acetone, potassium

[3]Samuel, G., R. J. Best and J. G. Bald, (1935). *Ann. Appl. Biol.*, 22 : 563-574.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

permanganate, hydrogen peroxide, formalin and phenol have been studied.

It was found that the pH-infectivity of the virus lies between 2.9 and 7.4.

ACKNOWLEDGEMENT

Thanks are due to Dr. R. S. Vasudeva, formerly Head of the Division of Mycology and Plant Pathology, for his keen interest in the work.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTS ON PHYSIOLOGIC SPECIALIZATION IN PUCCINIA PENNISETI ZIMM.

G. G. DALELA* AND S. SINHA

(Accepted for publication December 2, 1963)

Basu—Chaudhary and Sinha (1955) tested 20 varieties of *bajra* (*Pennisetum typhoides* Stapf & Hubb.) in seedling stage with several samples of *Puccinia penniseti* Zimm. collected from four distantly situated places in India and noted slight differences in the infection reactions, thus giving some indication of physiologic specialization in the parasite although all the varieties of the host proved susceptible. Ramakrishnan and Sundaram (1956) tested 32 bajra varieties in seedling as well as adult stage with the local (Coimbatore) rust and found all of them susceptible except the variety PT 814/3 which was fairly resistant; further on the basis of certain characteristic symptoms they described two races of the rust which were able to infect all the bajra varieties employed by them. The differential host in their work was a cross between *Pennisetum typhoides* Stapf & Hubb. and *P. purpureum* Schum. No reaction key was, however, prepared by them but they noted the disease intensity on each variety as determined by the modified Cobb Scale.

In the present investigation normal rust reactions of each of the 42 varieties of bajra (improved and stable ecotypes), 22 different from those used by Ramakrishnan and Sundaram (1956) were tested with ten samples of the rust collected from various localities, in an attempt to further determine the state of physiologic specialization in the parasite. Further, the reactions of mono-aecial cup infections were also studied to study the segregation of races, if any.

EXPERIMENTAL

Rust samples were collected from fields round about the district of Agra and also obtained from Coimbatore, Hyderabad., Kanpur, Lakhimpur Kheri, Meerut and Poona through the courtsey of State Mycologists. Stock cultures of the rust were maintained on Agra local variety which proved to be highly susceptible to all the rust samples. The original stock of cultures was maintained at Agra during October to March and at Simla during April to September through the kind permission of the Head of Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Normal Rust Reactions : 42 varieties of bajra were obtained from several parts of the country and seedlings tested, following the usual

*Present address : Junior Specialist, Plant Pathology Section, Department of Agriculture, Rajasthan, Jaipur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

technique for rust infection in glasshouses. Care was taken that the rusts from different places were always kept separate from one another in compartments partitioned by double muslin curtains. The rust reactions were recorded two weeks after inoculation following the rust reaction types recognized for wheat rust by Stakman and Levine (1922), as no key is available for *Puccinia penniseti* nor are the differentials known.

All the varieties of bajra were found to be susceptible to the rust. Only slight differences in the infection types were observed which were not distinct.

Mono-Aecial-Cup-Culture Reactions: Because no distinct variations were noticed in the infection reactions of the rust samples it was considered desirable to see if the physiologic races get segregated in the formation of aeciospores, possibly through hybridization. Aecial cups were produced on the alternate host (*Solanum melongena* L.) by infecting it with germinating teliospores. On the appearance of aerial cups, but before their opening, they were picked up with a sterilized needle and each of them was crushed separately on a piece of cellophane paper under a lens. Bajra seedlings of Agra local variety were then inoculated with these aeciospores. The infections thus obtained were designated as mono-aecial cup cultures or single aecial cup cultures. As a preliminary study only eight varieties of bajra were inoculated with aeciospores of each mono-aecial culture following the usual technique for rust infection and infection types recorded after a fortnight.

All the eight varieties of bajra tested show the same 3 and 3-4 types of reaction when inoculated with mono-aecial cup cultures. This indicates that no hybridization or segregation of races takes place. Certain varieties give 3 type of reaction with the stock culture and some of the mono-aecial cup cultures and 3-4 type with the other mono-aecial cup cultures. Nevertheless, the reaction types produced on bajra varieties by the stock cultures as well as by mono-aecial cup cultures were similar to a great extent. The vigour of infection, however, varied in some of the mono-aecial cup cultures.

Because no separation of races is evident it is likely that no hybridization in this rust occurs during the formation of aecia which is further confirmed by the fact that pycnia do not function and the rust is homothallic (Dalela and Sinha, 1962).

SUMMARY

Rust reactions of ten samples of *Puccinia penniseti* collected from Coimbatore, Hyderabad Dn., Lakhimpur Kheri, Kanpur, Poona, Meerut (2 localities) and Agra (3 localities) were studied on 48 varieties of bajra. The reaction types recognized for wheat rust by Stakman and Levine (1922) were followed. The possibility of races getting segregated during hybridization, if any, was checked by inoculating bajra varieties with mono-aecial cup cultures

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

produced on *Solanum melongena*, the alternate host. All these experiments were done in a glasshouse with the usual precautions in rust cultures. All the varieties tested were found to be susceptible while no segregation of races could be detected indicating that no hybridization occurs in the rust.

ACKNOWLEDGEMENTS : The authors are grateful to the Economic Botanists of various States and the Head of the Division of Botany, Indian Agricultural Research Institute, New Delhi for supplying seeds ; to various State Mycologists for obtaining rust collections; to the Head of the Division of Mycology and Plant Pathology, I.A.R.I., New Delhi and the Staff at the Rust Research Sub-station, Simla for maintenance of the rust cultures during summer months and to the Scientific Research Committee, Government of Uttar Pradesh for grant-in-aid.

Botany Department,
Agra College, Agra.

REFERENCES

BASU-CHAUDHARY, K. C. AND S. SINHA (1955) Physiologic specialization in *Puccinia penniseti* Zimm. on *Pennisetum typhoides* Stapf & Hubb. *Agra Univ. Jour. Res.* (*Sci.*), **4** : 575-578.

DALELA, G.G., AND S. SINHA (1962) Homothallism in *Puccinia penniseti* Zimm., a long cycled rust and the function of pycnia. *Indian Phytopath.*, **15** : 156-161.

RAMAKRISHAN, T. S. AND N. V. SUNDARAM (1956) Further studies on *Puccinia penniseti*. *Proc. Indian Acad. Sci.* **B**, **3** : 190-196.

STAKMAN, E. C. AND M. N. LEVINE (1922) The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. *Minn. Agr. Expt. Sta. Tech. Bull.* 8, 1922.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ASCOMYCETES ON CELASTRUS PANICULATA WILLD. II.

S. T. TILAK

(Accepted for publication December 15, 1963)

In the earlier paper the author (1963) has described two new species and a new host record for an ascomycetous fungus on *Celastrus paniculata* Willd. Further studies revealed the occurrence of two more Ascomycetes on the same host. These are described here as new to science on the basis of host specificity and comparative morphological studies.

Tympanopsis celastrina spec. nov.

Ascocarpi erumpentes, superficiales, collabentes ad maturitatem et liberantes ascos, 180—200μ, parietibus gracilibus constantibus e cellulis in pauca strata tenuia dispositis ; strato exteriore crasso. *Asci* plures implentes medium, pedicellati, unitunicati, aparaphysati, 38-45μ. *Ascosporae* plures, typice allantoideae, 1—2.8μ, hyalinae, demum olivaceae vel luteolae.

Ascocarps erumpent, superficial, collapsing at maturity releasing asci, measuring 180-200μ, wall thin, consisting of few layered thin walled cells while the outer layer is comparatively thick. *Asci* numerous filling the centrum completely, aparaphysate, pedicillate, unitunicate, measuring 38-45μ. *Ascospores* numerous allantoid or sausage-shaped, measuring 1-2.8μ, hyaline first, later changing to olive or yellowish in colour.

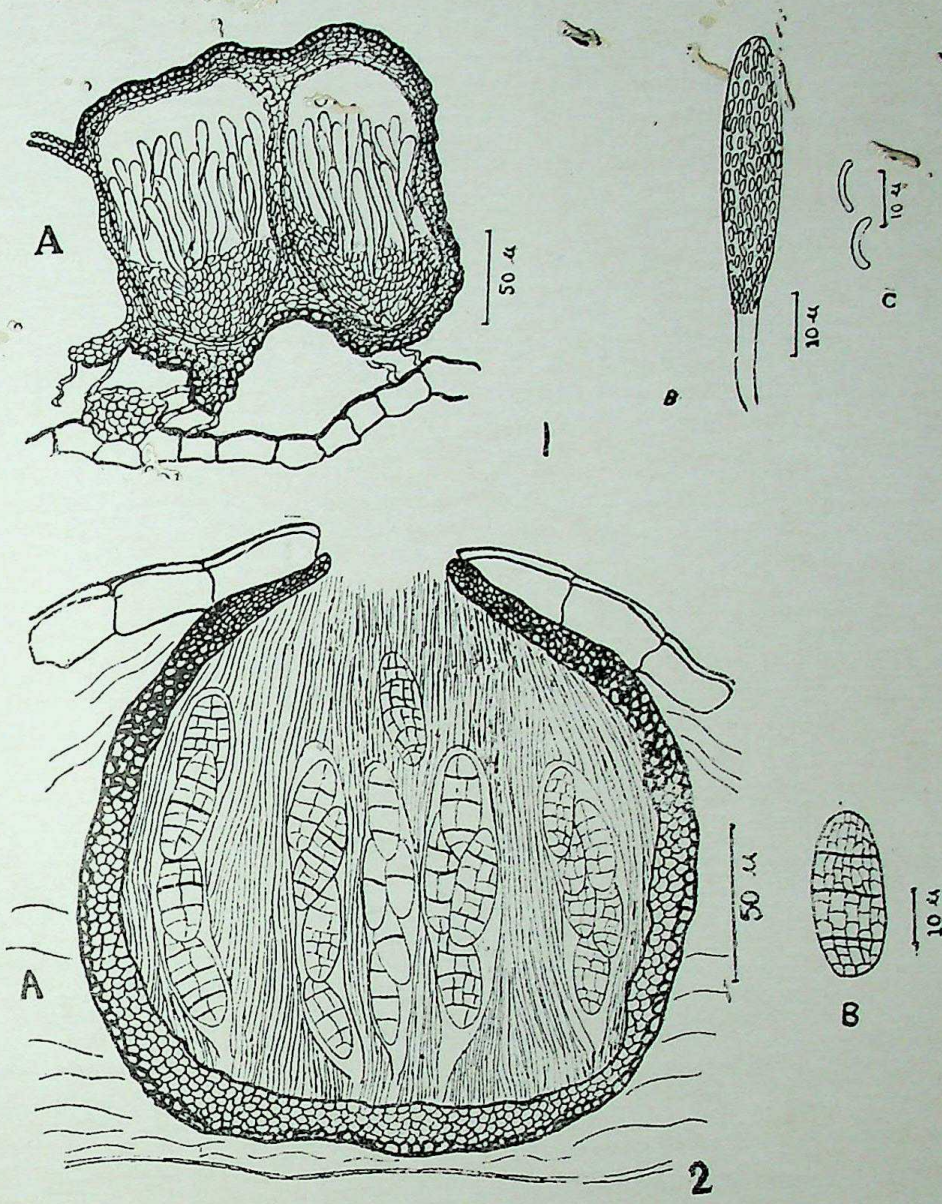
Saprophytic on dead stems of *Celastrus paniculata* Willd., collected at Daulatabad 26 March, 1963 deposited in Marathwada University under No. 106 (type).

The genus *Tympanopsis* had remained unrepresented in the Indian flora until the author reported it (1960) as occurring on dried stems of *Duranta plumeri* Facq. It has been previously reported under various names like *Coronophorella* V. Hoehnel (1909) *Eucanthe* Theisson (1907) *Fitzpatrickia* Ciferri (1928) *Scortechenia* Saccardo (1891) and *Teratonema* Sydow (1917). All these names have now been made synonyms of the genus *Tympanopsis* Starb. by Müller and Arx (1955).

The present species is typical in having allantoid ascospores similar to *Nitschkea* Otth and ascospores are numerous. Thus it is felt that the limitations of the various allied genera are overlapping and often lead to confusion. According to the Saccardian concept, the genus is paraphysate but recent investigations by Müller and Arx (1955) and by the author (1960) clearly suggest its aparaphysate nature. However the present collection differs in having numerous ascospores and thus stands distinct by itself. However on broad basis it has been refered to the genus *Tympanopsis*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



1. *Tympanopsis celastrina* A. Section through ascocarp. B. Ascus. C. Ascospores. 2. *Julella celastrina*. A. Section through ascocarp. B. Ascospore.

Ascocarpi globosi vel subglobosi, immersi in plantae hospitis textus, dispersi, 280-400 × 320-430μ., parte superiore ascocarporum efformante clypeum cum epidermide, et constante e cellulis crasse parietatis ; parte inferiore constante e cellulis graciliter parietatis. *Ostiolo* paulum elevato supra et diametiente 30-50μ. *Asci* cylindrici recti vel paulum curvati bitunicati, primo octospori, ad maturitatem 3-5 spori. *Asci* 116-205 × 20-26μ. *Paraphysoideae* plures emergentes ex textu basali stromatis et crescentes ad ostiolum filiformes. *Ascosporae* fusiformes vel elliposideae muriformes, irregulariter biseriatae initio, tum uniseriatae, 43-58 × 14-20μ. *Ascosporae* initio transverse tantum septatae septis tribus distinctis, posteae vero septa longitudilia exhibentur et sporae evadunt muriformes et unguttulatae.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Ascocarps globose or subglobose, immersed in host tissue, scattered, measuring from 280-400×320-430 μ; upper part of ascocarps forming clypeus with the epidermis and consisting of thick walled cells, the lower part of thin walled cells. *Ostiole* slightly raised above, measuring 30-50μ in diameter. *Asci* cylindrical, straight or slightly curved, bitunicate, eight spored in the initial stages later at maturity contain only 3-5 ascospores. *Asci* measure 116-205×20-26μ. *Paraphysoids* numerous starting from basal stromatic tissue and grow towards ostiole, filiform. The *ascospores* are fusiform to ellipsoid, muriform, irregularly biseriate in the beginning later changing to uniseriate, measuring from 43-58× 14-20μ. *Ascospores* in the intial stages transversely septate and the three septa are distinct, later longitudinal septa are formed and the spores become muriform and unguttulate.

On dead stems of *Celastrus paniculata* Willd., leg S. T. Tilak at Daulatabad 26 March, 1963 and deposited in Marathwada University Herbarium under No. 107 (type).

The genus *Julella* Fabre (1878) has been recently described from India by Bose (1962). The present species resembles the genus *Peltosphaeria* Berk. in having a clypeate ascocarp, but differs in possessing smaller spores which are hyaline.

Besides being a new species it forms a new addition to the generic list of Maharashtra State.

The type specimens have been deposited in the Herbaria of Marathwada University and Commonwealth Mycological Institute, Kew, England.

SUMMARY

The paper deals with two new species of ascomycetous fungi *viz.*, *Tympanopsis celastrina* and *Julella celastrina* on *Celastrus paniculata* Willd.

ACKNOWLEDGEMENT : The writer is grateful to Marathwada University for facilities and to Rev. Dr. H. Santapau for the latin diagnoses.

Department of Botany,
Marathwada University, Aurangabad.

Bose, S. K., *Indian Phytopath.*, **11** : 124-144 (1962)
Müller, E. and J. A. Von Arx., *Phytopath.*, **24** : 364 (1955)
Tilak, S. T., *Mycopath. et Mycol. Appl.*, **18** : 100-106 (1960)
Tilak, S. T., *Mycopath et Mycol. Appl.*, **21** : 60-63 (1963)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

A note on the prevalence of Meloidogyne spp. in vegetable crops :— C. L. SETHI, J. S. GILL AND G. SWARUP, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—A survey in the cultivated fields around Delhi for root knot nematode infestations was started in 1956. Roots of weeds and vegetables were collected and preserved in 5 per cent formalin for later detailed examination. Recently, Swarup ([1,2]) reported certain weeds and vegetable crops as hosts of *Meloidogyne* spp., the main species involved being *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949. This is a further report of the various species of *Meloidogyne* isolated from some crops and weeds.

Females were teased out from each plant specimen and the posterior portion was given a neat cut with surgical "eye" knife for study of the morphology of the perineal patterns. Sections of as many females as available were cut for this purpose and the key given by Taylor *et al.*,[3] was followed for identification. The various host crops examined and the species of *Meloidogyne* encountered are given in the Table below :

TABLE : Prevalence of *Meloidogyne* species in vegetable crops and weeds around Delhi

Name of host	*Meloidogyne* species encountered
Luffa cylindrica (Smooth gourd)	*M. incognita*
	M. javanica
L. acutangula (Ridge gourd)	*M. incognita*
L. aegyptica (Sponge gourd)	*M. incognita*
Solanum melongena (Brinjal)	*M. javanica*
	M. arenaria Neal, (1889) Chitwood, 1949.
	M. thamesi Chitwood *et al.*, 1952.
	M. incognita
Abelmoschus esculentus (Bhindi)	*M. arenaria*
	M. thamesi
	M. incognita
	M. javanica
Lycopersicon esculentum (Tomato)	*M. incognita*
	M. javanica

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE (Contd.)

Name of host	*Meloidogyne* species encountered
Dolichos lablab (Beans)	*M. arenaria*
Cucumis sativus (Cucumber)	*M. javanica*
Spinacea oleracea (Spinach)	*M. javanica*
Phaseolus radiatus (Green gram)	*M. incognita*
Trachysperma ammi (Ajwain)	*M. incognita*
Impatiens balsamina (Balsam)	*M. arenaria*
Corchorus acutangulus	*M. incognita*
Celosia sp. (Cockscomb)	*M. incognita* *M.* sp.
Digera sp.	*M. javanica*
Eragrostis pilosa	*M. incognita*
Vernonia cinerea	*M. incognita*
Vigna sinensis (Cowpea)	*M. javanica*
Amaranthus viride	*M. javanica*
Capsicum frutescens (Chillies)	*M. incognita*

It may be observed that in some cases more than one species of *Meloidogyne* was involved. In most of the cases of multiple infection single plants were found to be infected with more than one species of root knot. In *Bhindi* however, the infestations recorded were from different plants collected in different localities. There was no apparent difference in size of galls produced by different species of root knot nematodes. The host attacked appeared to be the determining factor in the size of galls produced. For example, *M. javanica* produced comparatively smaller galls on *Cucumis sativus* than on *Spinacea oleracea*. Similarly the galls produced by *M. arenaria* on *Impatiens balsamina* were bigger than those produced on *Dolichos lablab*. *M. thamesi* is recorded for the first time from India, and *Eragrostis pilosa* is a new host record.

The authors are indebted to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, I.A.R.I., New Delhi for going through the manuscript critically.

[1]Swarup, G. *Proc. Hort. Res. Workers Conf.*, Simla, (1959).

[2]Swarup, G. *Indian Phytopath.*, **15** : 228-230, (1962).

[3]Taylor, A. L., V. H. Dropkin & G. C. Martin, *Phytopathology*, **45** : 696-699, (1955).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A new Sorosporium species from Assam.—A. K. ROY*, Division of Mycology, Department of Agriculture, Jorhat, Assam.—During last June-July, the author made a few collections of smut on *Andropogon aciculatus* Retz. (=*Chrysopogon aciculatus* Trin.). The entire panicle was destroyed and replaced by a long slender sorus covered by a dirty white pseudomembrane in the early stage repturing to expose a long whip-like columella at maturity after the spore mass was shed off. The spore mass was brownish black and dusty containing isolated spores. Examination of a young sorus in serially cut sections as suggested by Thirumalachar and Pavgi[1] revealed the development of semi-opaque spore balls consisting mostly of fertile spores and a few sterile cells in their core. These observations indicated the smut belonged to the genus *Sorosporium*.

Two species of *Sorosporium* namely, *S. andropogonis-aciculati* Petch and *S. azmati* Mundkur have been recorded earlier on this host. Comparison of morphological characters of the sorus, spore balls and the spores of these species showed no resemblance with those of the species under study and hence it is proposed to describe it as new.

Sorosporium assamicum sp. nov.

Sorus, qui totam destruit inflorescentiam, primo includitur pseudomembrana albida papyracea constante e cellulis doliiformibus, (10-) 15—18×6.5—8 (-10) μ; portio apicalis explodit membranam et emergit ut flagellus longum nigrumque, quod est character princeps; pars inferior remanet in folio, membrana vero dissecata pendit deorsum. Flagellum ut plurimum 4-7 cm. longum, potest etiam attingere 11 cm., 0.25-0.50 mm. diam. curvatum, raro duplex. Sporarum massae plus minusve globosae, laxe unitae, fere opacae, 81—146μ diam., multisporae. Sporae brunneae, sub-globosae vel subellipticae, vel etiam paulum angulares, episporio 1.5μ crasso, levi, diametientes (11.5-) 13—16μ, ellipticae vero sporae attingere possunt etiam 19.5μ longit.

Sorus destroying the whole inflorescence, is at first enclosed by a whitish papery pseudo-membrane consisting of barrel-shaped cells, measuring (10-) 15—18×6.5—8 (-10)μ and the apical portion comes out bursting the membrane as a long black whip. The lower portion still remains inside the boot leaf and the shredded membrane hangs downwards. The whip is generally 4-7 cm. long, may be upto 11 cm. and 0.25-0.50 mm. in diameter, curved, rarely two whips may also be present. Spore balls more or less globose, loosely united, almost opaque, 81—146μ in diameter, many spored. Spores brown, subglobose, to sub elliptic or with slight angles, epispore 1.5μ thick (average), smooth and measure (11.5-) 13—16μ in diameter, elliptic spores may be upto 19.5μ in length.

Hab. : In the inflorescence of *Andropogon aciculatus* Retz. June-July, 1963, Borbheta, Assam, leg. A. K. Roy, deposited in the *Herbarium Cryptogamie Indiae Orientalis*, New Delhi, accession No. 27958 (type).

*Mycologist, Assam, Jorhat.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

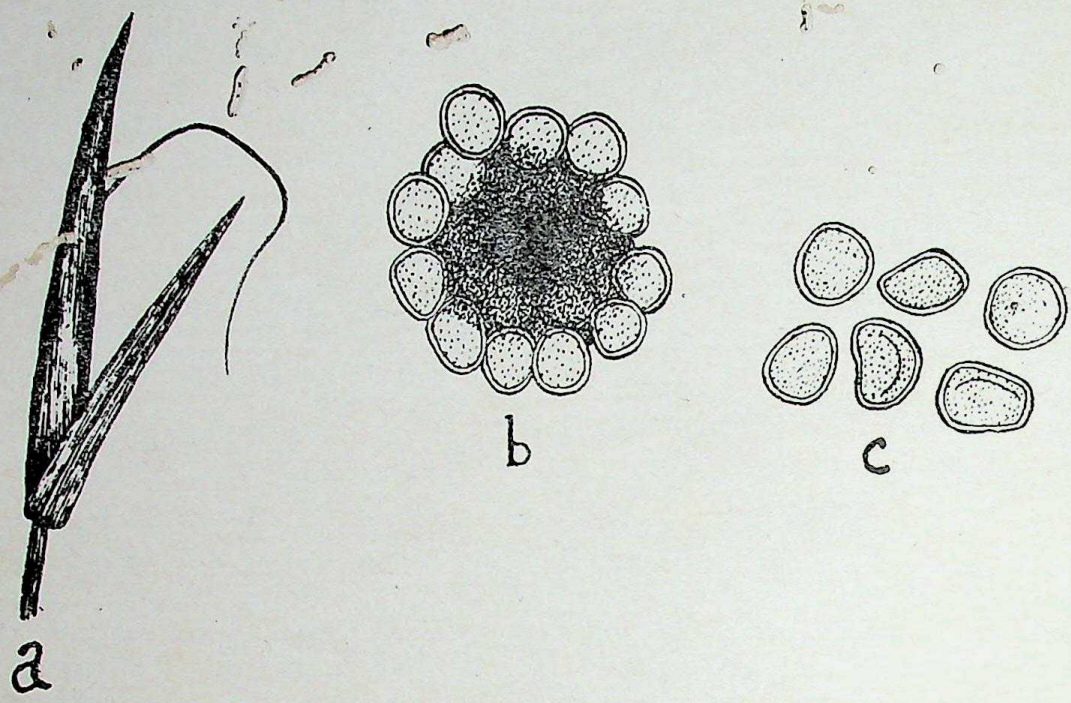


Fig. 1. Sorosporium assamicum : a—Infection ; b—spore ball × 310; c—Spores × 310.

ACKNOWLEDGEMENT : My thanks are due to Rev. Fr. Dr. H. Santapau, Director, Botanical Survey of India, for kindly rendering latin diagnosis of the new species and to Dr. M. S. Pavgi, College of Agriculture, Banaras, Hindu University, for kindly examining the specimen and critically reviewing the manuscript.

[1]Thirumalachar, M. J. and M. S. Pavgi, *Mycopathologia et Mycologia Applicata*, 7 : 282-290, (1953).

A new Physiologic race of Puccinia striiformis West. in India.—S. K. SHARMA AND SHEODHAN SINGH.—Indian Agricultural Research Institute, Plant Pathological Sub-station, Simla.—During the analysis of yellow rust samples from 1962-63 crop, a collection on barley variety K 40 from Kanpur (U.P.) revealed the presence of a new race in the country not previously reported by Mehta[1], and Prasada and Lele[2]. Reactions produced by the single spore culture of the rust sample on International differential varieties resemble those of race 57 (Fuchs[3]) originally reported from England on *Hordeum murinum* by Manners[4] as race M. The reactions of this race and race 57 are presented in the Table below for the sake of comparison.

It is seen from the Table that all the wheat varieties are resistant and only one variety of barley, *i.e.*, Fong tien is susceptible to this race. Even Agra local wheat, which is susceptible to all the physiologic races of the rust in India, is resistant to this race, so that the culture has to be maintained on a local barley grown commonly at Agra. The race appears to be specific on barley and, therefore, it may be necessary to test its pathogenicity on important barley material in the country.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE

Locality : Kanpur U.P.
Original Host : Var. K. 40

Stock Collection or Isolation	Michigan Amber	Ble Rouge D'ecosse	Strubes Dickkopf	Webster	Holzapfel's Früh	Vilmorin 23	Heines Kolben	Carstens V	Spaldings Prolific	Rouge P. Barbu	Chinese 166	Triticum Dic. Tric.	Barley Fong tien	Heils Franken	Petkuser Rye	Agra Local Wheat
Fong Tien 3-4	0 or 0-2	0	0	0	0	0 or 0-2	0 or 0-2	0	0	0	0	0 or 0-2	3-4	0	0	0
Race 7	0	0	0	0	0	0	0	0	0	0	0	0-1	3	0	0	0

ACKNOWLEDGEMENT : Grateful thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, I. A. R. I., New Delhi-12 for his encouragement and to Dr. R. Prasada for his keen interest during the course of these studies.

[1]Mehta, K. C., *Sci. Monogr. Coun. agric. Res., India*, **14**, (1940).
[2]Prasada, R. and V. C. Lele, *Indian Phytopath.*, **5** : 128-129, (1952).
[3]Fuchs, Eva, *Nachr. Bl. Pfesch Dienst* (*Braunschweig*) **8** : 87-93, (1956.)
[4]Manners, J. G., *Ann. appl. Biol.*, **37** : 187-214, (1950).

Lipolytic activity of some Xanthomonads :—A. C. PADHYA, W. V. KOTASTHANE AND M. K. PATEL.—Microbiology Department, S. B. Garda College and B. P. Baria Science Institute, Navsari, Gujarat State.—Many workers have evinced considerable interest in the lipolytic activity of the phytopathogenic bacteria, especially of the *Xanthomonas* group. One of the reasons for this may be that among *Xanthomonas* spp. (about 125) except for the host specificity in general and fermentation of salicin, lipolytic activity appears to be the only other distinguishing feature. Starr and Burkholder[3] investigated the lipolytic activity of 24 and found 21 to be lipolytic. We have now examined 14 more species, along with *X. citri* (Hasse) Dowson and *X. malvacearum* (Sm.) Dowson for comparison.

Organisms tested : (i) *X. alangi* Padhya and Patel, (ii) *X. bauhiniae* Padhya and Patel, (iii) *X. citri* (Hasse) Dowson, (iv) *X. corianderi* Sriniv., Patel and Thirum., (v) *X. durantae* Sriniv., Patel and Thirum., (vi) *X. ionidi* Padhya and Patel, (vii) *X. lantanae* Sriniv., Patel and Thirum., (viii) *X. malvacearum* (Smith) Dowson, (ix) *X. nakataecorchori* Padhya and Patel, (x) *X. Pateli* Desai and Shah, (xi) *X.*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

poinsetticola Patel, Bhatt and Kulkarni, (xii) *X. spermacoces* Sriniv., Thirum. and Patel. (xiii) *X. Thirumalachari* Padhya and Patel, (xiv) *X. tribuli* Sriniv., Thirum. and Patel, (xv) *X. vesicatoria* (Doidge) Dowson and (xvi) *X. vitis-trifoliae* Padhya and Patel.

Fats used : A. *Oils* : (i) Bassia (*Bassia latifolia* Roxb.), (ii) Coconut (*Cocos nucifera L.*), (iii) Crassa *Cressa cretica L.*), (iv) Groundnut (*Arachis hypogaea* L.), (v) Castor (*Ricinus communis* L.) (vi) Sesame (*Sesamum orientale* L.) (vii) Karanj (*Pongamia glabra* Vent). B. Other : (viii) Dalda (hydrogenated oil), (ix) *Ghee* (Clarified butter), (x) Butter and (xi) Tributyrin.

Medium used : 10 g. Peoptone; 5 g. Yeast extract ; 25 ml. oil emulsion (25 per cent); 50 ml. alcoholic solution of Nile blue chloride (0.3 per cent); 20 g. agar. Distilled water to make up to 1000 ml. pH—7.2.

Indicator : Nile blue had been substituted for Spirit blue (Starr[2], and Night Blue Cabral[1], Lipolysis is indicated by colourless zone around the bacterial growth.

Oil emulsion : A rough emulsion was made by titurating 5 ml. of oil with 20 ml. of gum arabic solution (2.5 per cent). A smooth emulsion was effected by vigorous and continuous stroking for about 5 min. with the help of a hypodermic syringe.

The medium was autoclaved at 15 lb. pressure for 15 min. and stored in a dark cool place.

Plates containing 20 ml. of the medium were inoculated with 36-40 hr. cultures grown in nutrient dextrose broth. After incubation at 27°—28°C, for a week, the plates were examined for lipolysis.

A critical examination of results showed that all the *Xanthomonas* spp. noted here are lipolytic. Lipolytic activity does not appear to be affected by the nature of fats. Tributyrin gives a considerably large zone of activity by almost all the organisms. Pongamia oil, perhaps because of some toxic constitution, interferes with growth of all the organisms.

In view of these findings, it is suggested that lipolytic activity may form part of routine tests for *Xanthomonas*, in determining the systematic position at special level.

---

[1]Cabral, R. V, de G. *Dissertation for the Ph. D. Degree*, Cambridge University.(Unpublished, 1944).

[2]Starr, M. P. *Science*, **93** : 333-334. (1941).

[3]Starr, M. P. and W. H. Burkholder, *Phytopathlogy*, **32** : 592-604, (1942).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Occurrence of Periconia sacchari Johnst. and Phyllosticta sacchari Speg. on Sugarcane in Assam.— A. K. ROY*, Division of Mycology, Department of Agriculture, Jorhat, Assam.—During a survey of sugarcane diseases in Assam, the author made two collections of *Periconia sacchari* Johnst. and *Phyllosticta sacchari* Speg. respectively from Borbheta (near Jorhat). The former was first described from Puerto Rico and the latter from Tucuman, Argentina. *Periconia sacchari* was collected on half dried leaves and *Phyllosticta sacchari* on characteristic ring spot on leaves. The following are brief descriptions of the fungi.

Periconia sacchari Johnst. :—

Occurs on entire dried lamina as greyish black mould. Conidiophores amphigenous, copious, arise singly or rarely in groups of two, erect, stout, fuscus, lighter at the tip, sparingly septate (generally 3-4 septa), the apex may shortly be branched, measure $200-390 \times 12-16\mu$ and bear a tuft of conidia at the tip. Conidia sessile, brown, oval, finely verrucose and measure $12-22 \times 7-11\mu$.

Phyllosticta sacchari Speg. :—

Small, oval, purplish, amphigenous spots appear on the leaves which become bigger and straw coloured at the centre, the margin is limited by a purplish band. Pycnidia arise on dried straw-coloured portion, minute, globose, membraneous, cellular, ostiolate and measure $68-108\mu$ in diameter. Spores abundant, hyaline, thin-walled, elliptic, generally bi-guttulate and measure $7-9 \times 2.4-3.2\mu$. Conidiophores incospicuous, simple. The spores, in this case are somewhat longer than the original description but all other characters agree.

*Mycologist, Assam, Jorhat.

Control of leaf spot disease of spinach caused by Cercospora beticola. :—R. L. MATHUR AND S. P. SEHGAL. Plant Pathology Section, Department of Agriculture, Jaipur, Rajasthan.—Smooth leaf type Spinach (*Beta vulgaris* var. *bengalensis*) suffers from a leaf spot disease caused by *Cercospora beticola* Sacc. (Sehgal and Mathur,[1]). As this vegetable is primarily grown for its leaves, spotting and discolouration results in decreased yields and market value. Four fungicides *viz.*, 5 : 5 : 50 Bordeaux mixture, 0.25 per cent Blitox (Copper oxychloride), 0.25 per cent Crag fungicide 658 (Copper zinc chromate) and 0.25 per cent Fungimar (Cuprous oxide) were tried for the control of the leaf spot disease and the results are presented in this note.

Seeds were sown in 9" pots and after 40 days, the seedlings were thinned out to 10 per pot. Spraying of the fungicides in the required concentrations at the rate of 100 gallons solution per acre *i.e.*, 4.5 ml. for each pot was carried out immediately after thinning with an atomiser, Six pots were treated with each fungicide. Two days after

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spraying, when the plants were six weeks old, they were inoculated with a heavy spore suspension of the fungus prepared from fresh diseased leaves. Two sets of 3 pots each with no fungicidal treatments were kept one with inoculated and the other with uninoculated plants as controls. After a fortnight, treated plants in three pots out of six were given a second fungicidal spray at the same rate. The pots were kept under moist chamber for 48 hours after inoculation. Observations on the incidence of disease were noted after 4 weeks of inoculation. Each leaf was examined and rated on the basis of the number of spots developing on it. The scale of numerical ratings was 0; for 0; 0.5 for 1-5; 1.0 for 6-10; 1.5 for 11-15; 2 for 16-20; 2.5 for 21-25 and 3.0 for 26 and more spots per leaf. About 30 spots covered nearly the entire leaf surface. The infection index was calculated by the following formula (Chester.[2]) :

$$\text{Infection Index} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of inoculated leaves} \times 3}$$

The efficiency of the different treatments for disease control was calculated as :

$$\frac{\text{Infection Index in control-Infection index of treatment} \times 100}{\text{Infection index of control}}$$

The details of the observations for different treatments are presented in Table 1.

TABLE 1 : Incidence of leaf spot disease of Spinach under different fungicidal treatments

S. No. Fungicides	No. of sprays	No. of leaves in 3 pots	Sum of numerical ratings	Infection index	Percentage efficiency of disease control
1. Bordeaux Mixture (5 : 5 : 50)	1	179	2.5	0.47	98.8
2. ,, ,, (,,)	2	187	0.5	0.09	99.7
3. Blitox (.25 per cent)	1	171	47.0	9.04	76.7
4. Blitox (-do-)	2	170	30.5	5.98	84.5
5. Crag fungicide (.25 per cent)	1	180	29.5	5.46	85.9
6. Crag fungicide (,,)	2	176	20.0	2.27	94.1
7. Fungimar (0.25 per cent)	1	186	18.5	3.69	90.5
8. Fungimar (,,)	2	171	9.0	1.75	95.5
9. Control (Inoculated)		163	190.0	38.85	
10. Control (Uninoculated)		124	0	0	

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

From the figures of percentage efficiency of disease control, it is apparent that the merit of fungicidal efficiency for one spray was in the order of Bordeaux mixture (98.8), Fungimar (90.5), Crag fungicide (85.9) and Blitox (76.7). Two sprayings have in each case showed better control of the disease than one. All the fungicides haved proved efficacious in controlling the disease to the extent of above 75 per cent. Two sprayings of Bordeaux mixture (5 : 5 : 50) on 6 and 8 weeks old plants at the rate of 100 gallons of per acre practically eliminated the disease and has been found to be the best that can be recommended for effective control.

The authors are grateful to Dr. N. Prasad, the then Plant Pathologist, Rajasthan for kind guidance and encouragement during the progress of work and to Shri Samrath Raj, R.A.S., Ex-Director of Agriculture, for facilities.

[1]Sehgal, S. P. and B. L. Mathur, *Sci, & Cult.*, **25** : 77. (1959).

[2]Chester, K. S. *Plant Dis. Reptr.* Supplement 193, (1950).

Seed transmission and epidemiology of the bacterial blight disease of rice in North India.—D. N. SRIVASTAVA AND Y. P. RAO, Divison of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—In an earlier publication, the authors[1] reported the isolation of a highly pathogenic strain of *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson, the incitant of bacterial blight disease of rice, from diseased rice plants obtained from the epidemic areas of Shahabad, Bihar. The bacterium killed rice seedlings within 14 days when spray inoculated. Paddy samples of 2 vaieties from Sambalpur district of Orissa showed 100 per cent seed infection by the bacterium.

Further studies have revealed 100 per cent seed infection by the bacterium in a paddy sample obtained from Shahabad, and 50 per cent in another one from Kanpur, Uttar Pradesh. The infection was present not only in the husk of the seeds but also in the endosperm.

The leaf isolate of the bacterium from Bihar was considerably more virulent than the seed isolate from Orissa. The virulence of the bacterium was appreciably reduced when subcultured on nutrient agar 4 times in 5 months; storing the cultures in mineral oil prevented this loss of virulence.

Infected seeds transmitted the disease to the seedlings when raised under conditions of high humidity. In the case of Shahabad seeds, all the seedlings raised in humid glazed chamber were killed within 3 weeks, those of T.90 from Sambalpur in 4 weeks, and in the case of Kanpur seeds, it took 5 weeks. When surface sterilized pieces of root, stem and leaf of the diseased seedlings were plated on nutrient agar, the bacterium was readily recovered in cultures in all the cases, indicating that the disease was systemic and was transmitted internally. The bacterium

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

could also be isolated in several cases from smears of guttation drops collectd from the leaf tips before manifestation of the disease. The fact that seedlings raised from the infected seeds outside the moist chambers did not develop the disease within 5 weeks shows that high atmospheric humidity was essential for transmission and development of the disease.

The magnitude of seed infection observed in the samples from Orissa, Bihar, and Uttar Pradesh presents an event unprecedented in phytopathological history. The situation calls for an all out drive to discover ways and means of fighting the disease.

The authors wish to acknowledge their grateful thanks to Dr. B. P. Pal, Director, Indian Agricultural Research Institue, for his keen interest and encouragement in this work. Thanks are also due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute for facilitating these investigations and for his keen interest in the work.

Seeds from Kanpur were kindly made available by Dr. H. K. Saksena, Professor of Plant Pathology, Government Agricultural College, Kanpur and those from Shahabad by Dr. H. P. Srivastava, Deputy Director of Agriculture, Bihar through Dr. K. N. Singh, Professor of Agricultural Extension at the Indian Agricultural Research Institute, New Delhi.

[1]Srivastava, D. N. and Y. P. Rao, *Indian Phytopath*, 16 : 393-394, (1963).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MANUSCRIPTS FOR INDIAN PHYTOPATHOLOGY

Contributors are requested to follow the instructions given hei while preparing their manuscripts.

MANUSCRIPT COPY : Mannscripts should be typed preferably on white sheets (size $8\frac{1}{2} \times 11$ inches) on one side of the page only with at leasts $1\frac{1}{4}$ inch wide margin on the left and $\frac{1}{2}$ inch on each of the remaining sides. All sheets in the copy should be of one size only. Even in cases where only a few lines are to be added, full size sheets alone should be used.

The entire manuscript should be clearly typed double-spaced including title, foot notes, quotations, references, and legends. The writing style should be simple and direct. Check up the copy for spellings, punctuations, diacretic marks, pagination, etc. All calculations, tables, figures, names, quotations, citations, etc. should be carefully verified before submission. Statistical data likewise must be approved by some one competent in Statistics.

Since tabular matter is expensive to print, it should be reduced to a bare minimum just necessary for substantiating the results. All tables, when included, should be consecutively numbered, and each table should have a heading stating concisely its contents.

The foot notes if any, should follow immediately after the line in which its reference appears and be made to appear separate by drawing lines across the page thus[1].

Foot note[1].

Instructions, if any, either to the Editor or Printer, should not be incorporated in the actual MS copy. These may be given on a separate unnumbered sheet.

The contents of the MSS should have brief headings and should broadly be given in the sequence thus: Title, (in capitals), name or names of authors, Introduction, Material and Methods, Results, Discussion, Summary, Acknowledgements, if any, Institutional Address (on left side of the page arranged flush with the margin), References, and Legends for figures.

To comply with the International Rules of Botanical Nomenclature (article 34) now in force, Latin diagnosis must be supplied with each new taxon proposed. It may be emphasised that this need not be a verbatim or literal translation of the full English description. An abbreviated Latin diagnosis giving essential characters only with a full description in English will be preferred. Unnecessary duplication (such as inclusion of collection data) in both the languages should be avoided.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ILLUSTRATIONS : The available space for text figures in *Indian Phytopathology* is 4 inches in width and $6\frac{1}{2}$ inches in length. These therefore, should be $4 \times 6\frac{1}{2}$ inches when reduced. As many of the figures as possible should be **grouped** and they should invariably be mounted close together leaving **no space in between** for reproduction as a single block. Mounting should be done on stiff white carboard. Use rubber solution for pasting the figures on the board, as it is not messy.

Illustrations should be numbered consecutively in the order in which they are to appear in the text. The numerical sequence should be maintained even in cases where both photographs and India Ink drawings are included. Thus there should be only one figure bearing any particular number.

For numbering avoid use of typewritten symbols, letters, etc. Instead it can be accomplished through a lettering device (a stencil, for example) or printed letters or numbers may be used for this purpose.

Because of high cost of half tone reproduction, (used mainly for photographs, photomicrograph, etc.), carefully made line drawings in India Ink will be preferred. One page of half tone illustration is permissible per paper or article or its equivalent.

The photographs and line drawings, graphs, charts, etc. should not be mounted or grouped together. The former are reproduced as half tone blocks while for the latter zinc-etching process of reproduction is used.

The drawings, done in jet black undiluted India Ink, should be bold enough for half or more reduction. Fine stippling consisting of small dots or dashes may be avoided as it often disappears in reproduction.

A scale may be added with each drawing or a group of drawings drawn at the same magnification. This is helpful for the printer to reduce the original figures to any desired size.

Original drawings will be preferred over their photostat copies since in the latter during reproduction, the background becomes murky and grey.

All unnecessary background and foreground in photographs should be trimmed. The trimming should be so done that the margins become rectilinear. Trimming which results in having grotesque or crooked margins should be avoided. Unless the edges are straight, the background has to be routed resulting in increasd cost. Each figure must have a legend. The legends should be typed in double space on a separate page or pages. These should not be pasted or attached to the figures. It may be noted that the legends go to the printer while the figures are sent to the engraver.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES : All references should be double-spaced throughout. These should be listed in the alphabetical order of the authors' names after the Institutional address at the end of the paper. These may be cited as foot notes when they are four or fewer in number. In the text the references should be referred by date thus : Chaudhury (1949) or (Chaudhury, 1949). A citation should give the name of the author (or authors), his/her (or their) initials, year of publication within parentheses, and then the full title correctly, followed by the name of journal (to be underlined), number of the volume, a colon and page numbers. The diacretic signs and capitalization either in the title or in the name of the journal should be precisely as in the original.

The names of the journals should be abbreviated as given in the 3rd Edition (1952) of the WORLD LIST OF SCIENTIFIC PERIODICALS. The Biological Council of England has issued : A LIST OF ABBREVIATIONS OF THE TITLES OF BIOLOGICAL JOURNALS. Authors not having access to the WORLD LIST may consult this before finally typing the references for their papers.

If an article has not been seen in the original, then that fact should be clearly stated. When a paper in the name of two or more authors is to be cited, only the initials of the first author are to come after the surname, thus : Mueller, E. and S. K. Bose and not Mueller, E. and Bose, S. K. While citing two or more references from the same Journals and/or of the same authors avoid using words : *Ibid.*, *Loc. cit.*, etc. and give the full citation.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS ABOUT INDIAN PHYTOPATHOLOGY

1.	Place of Publication	New Delhi-12
2.	Periodicity of its Publication	Quarterly
3.	Printer's Name Nationality Address	Shri S. N. Malhotra INDIAN M/s Kapoor Art Press, Karol Bagh, New Delhi.
4.	Publisher's Name Nationality Address	Dr. R. Prasada INDIAN Editor, Indian Agricultural Research Institute, New Delhi-12
5.	Editor's Name Nationality Address	Dr. R. Prasada INDIAN Indian Agricultural Research Institute, New Delhi-12
6.	Names and addresses of individual who own the newspaper and partners or shareholders holding more than one per cent of the total capital.	(Indian Phytopathological Society Registered No. S. 399)

I, Dr. R. Prasada hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated : 30th June 1964.

Sd/- R. Prasada
Signature of Publisher.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CIBA Pesticides

Dimecron — a systemic insecticide, based on Phosphamidon, is effective against sucking, chewing and mining insects. It is water soluble, odourless, does not taint and most crops are free from residues ten days after treatment.

Nuvan — an insecticide with extremely potent fumigant and contact action against household and storage pests as well as against chewing and mining caterpillars on field crops.

Thiocron — an acaricide cum insecticide of low toxicity with specific action against mites.

Cuman — an organic fungicide with excellent rain resistance, suitable for the control of diseases on fruit trees and field crops.

Cosan — a wettable sulphur preparation of colloidal particle size for the control of powdery mildew on fruit crops and against mites.

Copramat — a fungicide, based on a combination of Copper-oxychloride and ziram for the control of diseases on field crops.

For detailed information, please write to
CIBA of India Limited, Pesticides Division, P.O. Box 479, Bombay 1

JWT-C. 205 (A)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(*Contd. from 2nd cover*).

Phytopathological Notes

A note on the prevalence of Meloidogyne spp. in vegetable crops—*C. L. Sethi, J. S. Gill and G. Swarup* ... 69

A new Sorosporium species from Assam—*A. K. Roy* ... 71

A new Physiologic race of Puccinia striiformis West. in India—*S. K. Sharma and Sheodhan Singh* ... 72

Lipolytic activity of some Xanthomonads—*A. C. Padhya, W. V. Kotasthane and M. K. Patel* 73

Occurrence of Periconia sacchari Johnst. and Phyllosticta sacchari Speg. on Sugarcane in Assam—*A. K. Roy* ... 75

Control of leaf spot disease of spinach caused by Cercospora beticola—*R. L. Mathur and S. P. Sehgal* 75

Seed transmission and epidemiology of the bacterial blight disease of rice in North India—*D. N. Srivastava and Y. P. Rao* 77

Manuscripts for Indian Phytopathology 79

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1964

President	... ...	S. SINHA
Immediate Past President	... ...	T. S. SADASIVAN
Vice-President	... ...	N. PRASAD
Secretary-Treasurer	... ...	S. P. RAYCHAUDHURI
Joint Secretary	... ...	D. SURYANARAYANA

COUNCILLORS

Northern Zone	... ...	B. L. RENFRO
Mid-Eastern Zone	... ...	G. S. VERMA
Eastern Zone	... ...	U. N. MOHANTY
Central Zone	... ...	B. P. PATIL
Western Zone	... ...	K. G. NEMA
Southern Zone	... ...	S. B. LAL

EDITORIAL BOARD FOR 1964

R. PRASADA

Editor-in-Chief

S. P. RAYCHAUDHURI
M. J. THIRUMALACHAR
K. S. BHARGAVA
G. RANGASWAMY
KISHAN SINGH
D. N. SRIVASTAVA
M. M. PAYAK

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12 (India).

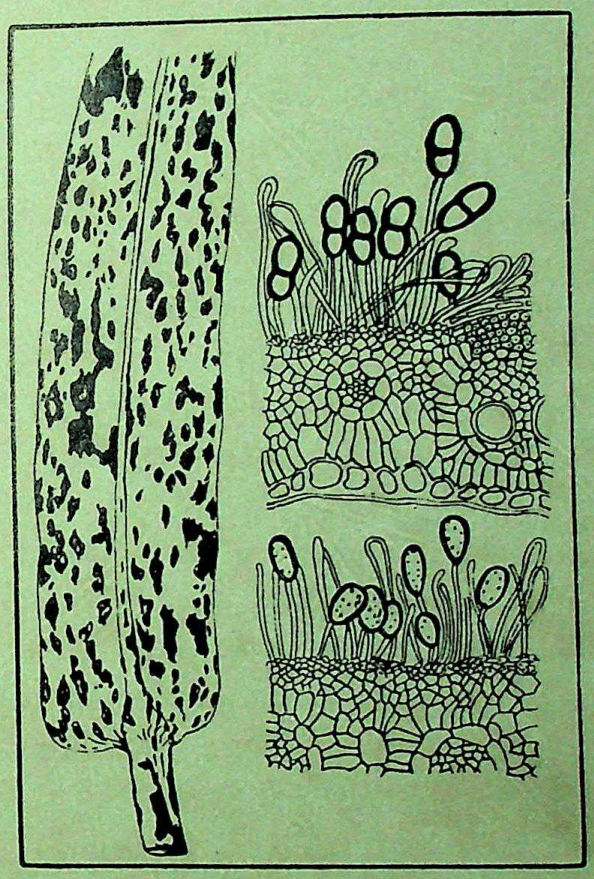
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVII **1964** *NUMBER 2*



PUBLISHED FOR

THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Study on eleven isolates of Phyllosticta rabiei (Pass.) Trot., the causal agent of Gram blight in the Punjab—*Shamsher Singh Aujla* 83

Root-knot of vegetables III. Factors affecting hatching of eggs of Meloidogyne javanica javanica (Treub, 1885) Chitw., 1949 and M. incognita incognita (Kofoid and White, 1919) Chitw., 1949 – *Gopal Swarup and K. J. Pillai* 88

Factors influencing larval hatching in the root-knot nematodes, Meloidogyne incognita (Kofoid and White., 1919), Chitwood, 1949, and M. javanica (Treub, 1885), Chitwood, 1949—I-EFFECT OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION—*Asad Ahmed and Abrar M. Khan* 98

Factors influencing larval hatching in the root-knot nematode Meloidogyne incognita (Kofoid and White 1919), Chitwood, 1949—II-EFFECT OF ROOT LEACHATES AND CERTAIN CHEMICALS—*Asad Ahmed and Abrar M. Khan* ... 102

Environmental conditions and false smut incidence in rice—*K. Manibhushan Rao* 110

Effects of varying water supply on the susceptibility on Gomphrena globosa L. plants to infection with potato virus X—*D. C. Sharma* 115

Effect of some vitamins on the growth of Fusarium oxysporum F. cumini isolates—*B. L. Mathur, L. C. Sharma and N. Prasad* 121

Studies on host range and histopathology of root-knot infections caused by Meloidogyne javanica—*M. Balasubramanian and G. Rangaswami* 126

Some observations on linseed wilt—*L. B. Goel and Gopal Swarup* 133

Natural infection of Oxalis corniculata L., the alternate host of Puccinia sorghi Schw., in India—*D. P. Misra and S. K. Sharma* 138

Further studies in the crown rust of oat in India—*D. P. Misra, S. K. Sharma, P. C. Joshi and Sheodhan Singh* ... 142

Ramularia areola Atk. on herbaceum cottons in Vidharbha (Maharashtra)—*M. R. Siddiqui and S. B. P. Rao* ... 146

(Contd. on 3rd cover)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDY ON ELEVEN ISOLATES OF PHYLLOSTICTA RABIEI (PASS.) TROT., THE CAUSAL AGENT OF GRAM BLIGHT IN THE PUNJAB

SHAMSHER SINGH AUJLA

(Accepted for publication April 7, 1964)

INTRODUCTION : The gram crop in the Punjab State is subject to a number of diseases, among which the Blight disease caused by *Phyllosticta rabiei* (Pass.) Trot., is highly destructive. The disease is now spreading to the plains of the Punjab and it is feared that it may become a limiting factor in the cultivation of gram in the Districts of Gurdaspur, Amritsar, Hoshiarpur, Jullundur, Ambala, Ludhiana and Patiala. In this respect, preliminary work indicated that the different isolates varied in pathogenicity. It was noticed that some isolates produced very severe infection and the others very mild infection on the same varieties. Luthra, Sattar and Bedi (1939) isolated six forms of the gram blight fungus. They also found that these forms, although morphologically different, were quite identical in pathogenicity. During the "Rabi" season of 1959-60, the gram blight disease appeared in an epiphytotic form and the resistant varieties also showed heavy infection.

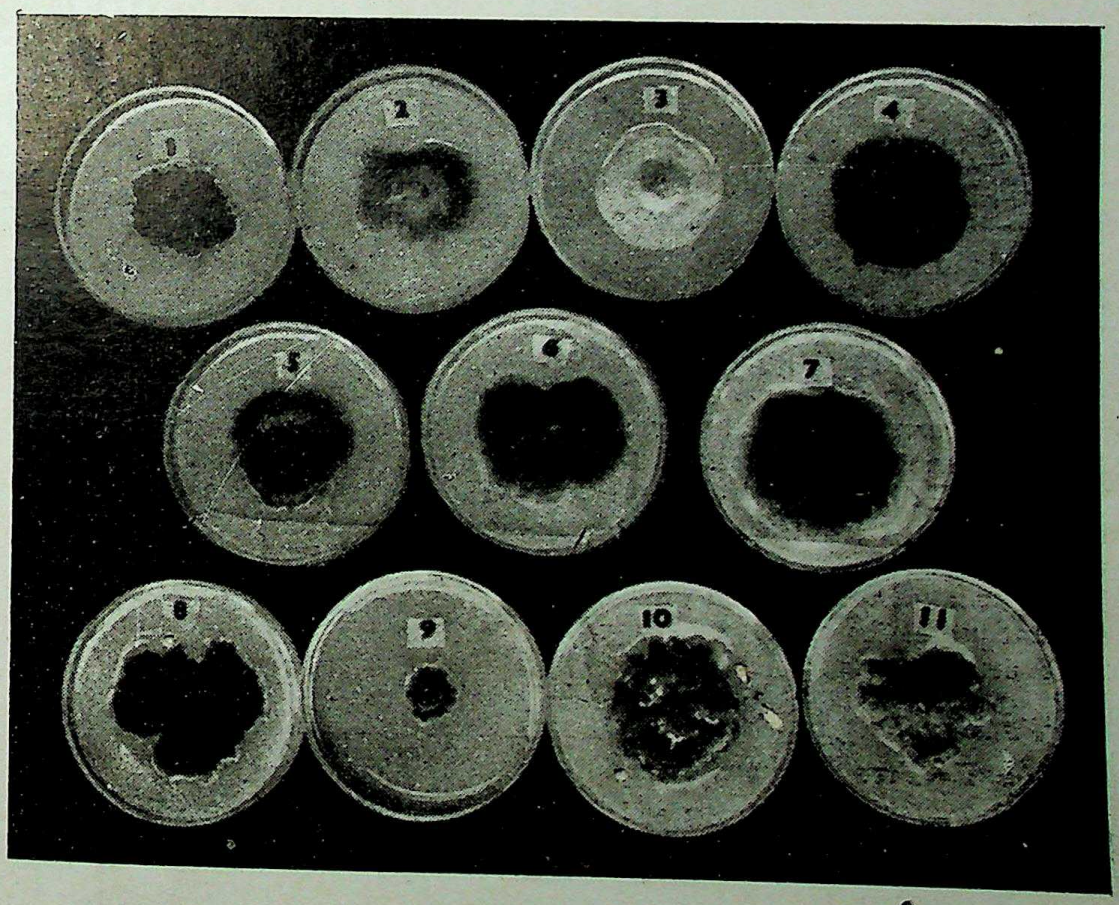


Fig. I : Showing colonies of eleven strains of gram blight fungus, when grown on Richard's solution agar at 9°C for 21 days.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL (a) : A large number of isolations was made from the leaves, stems and pods collected from different localities of the Punjab State. The purified isolates could be grouped into eleven main categories on the basis of their cultural characters. These main morphological characters, when grown on Richard's solution agar are given in Table 1.

TABLE 1 : Showing the average colony diameter, and other cultural characters of eleven isolates, on Richard's solution agar (17°-19°C) at the end of 21 days

Isolate No.	Colony diameter in mm.	Colour of colony	Size of Pycnospores in microns	Size of Pycnidia in microns
1.	35	Light mouse grey	—	—
2.	43	Light grey	8.9×4.2	214×195
3.	46	White	—	—
4.	47	Deep grey	13.0×4.5	245×239
5.	51	Mouse grey	10.7×4.2	169×155
6.	48	Pellucid grey	8.2×4.5	217×201
7.	52	Light grey	9.5×4.5	172×152
8.	49	Greyish	8.0×3.7	198×166
9.	24	Pink	10.5×4.5	175×156
10.	50	Jet-black	—	—
11.	38	Light grey	8.0×4.0	186×169

It is interesting to note that the range of linear growth rate varied from 24mm. to 52 mm. in some isolates. The size of the pycnospores and pycnidia also varied markedly in all the isolates.

(b) *Differentiation of different pathogenic races of* Phyllosticta rabiei : Fifteen differential varieties were selected out of a large collection of gram varieties. The dried-up mycelial mat method of inoculation evolved by Bedi (1949) was followed. The mycelial mats of all the eleven isolates were dried and used for the inoculation of varieties grown in separate plots. Each plot was enclosed with 7 to 8 ft. high "*sarkanda*" (*Saccharum spontaneum*) screens to protect them from infection from adjacent plots. A pathogenicity index (see below) was evolved and the results are presented in Table 2.

Pathogenicity Index :

F=Free from any infection

T=Traces or flecks of infection

L=Light type of infection in which immature pycnidia were seen

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2 : Reaction of fifteen varieties of gram to eleven isolates of *P. rabiei*

Variety	isolate 1	isolate 2	isolate 3	isolate 4	isolate 5	isolate 6	isolate 7	isolate 8	isolate 9	isolate 10	isolate 11
C 1	S	S	S	S	L	V.S.	S	S	S	V.S	V.S
Tehr an 32	S	S	L	M	S	M	S	V.S	V.S	V.S	V.S
7/120	S	S	S	S	S	V.S	S	V.S	L	M	S
1234	S	S	S	S	L	S	S	V.S	T	S	M
F. 8	F	L	T	L	L	M	L	V.S	M	L	T
C 104	S	S	S	S	S	S	V.S	V.S	L	S	M
Pb. 1	S	S	S	M	S	M	V.S.	V.S	L	S	M
14/15-6	S	S	S	S	S	V.S	V.S	V.S	M	S	V.S
621	S	S	S	S	S	V.S.	L	V.S.	S	V.S.	V.S
172/3	M	M	L	M	M	V.S.	V.S	V.S	M	L	M
81	S	S	L	S	S	S	V.S.	V.S	M	V.S.	M
235	S	S	L	S	S	S	S	V.S	M	S	M
246	L	L	F	M	S	L	F	V.S	L	M	L
150	S	S	L	S	S	V.S	S	V.S	V.S	S	M
Kadlay 1	S	S	M	S	S	L	S	V.S	V.S	V.S	S

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

M = Moderate type of infection in which fully mature pycnidia were formed and were scattered on the surface.

S = Severe infection in which the whole plant is covered with pycnidia, but the plant still living.

V.S = Very severe infection in which only the stalks of the gram plant are left behind.

It is possible to distinguish strains of the gram blight fungus on a set of different varieties which may be selected for this purpose. Isolate No. 1 produced no infection on F-8, while it attacked other varieties and the intensity of infection varied from moderate to severe infection. Thus, this race can be easily distinguished from the other ten races by its inability to attack F-8. Isolate No. 2 showed light type of reaction on F-8, moderate on 172/3 and light on 246 variety. None of the other isolates gave this triple reaction. Similarly, the other isolates gave interesting results which quite differed from one another in type of reaction. From these it is quite clear that a particular race of the fungus is responsible for breaking down the resistance of a resistant variety.

Summary

The blight disease of gram had been observed in the plains of the Punjab causing heavy losses during certain years. A large number of isolations from the affected gram plants which had been obtained from different localities revealed the presence of eleven distinct strains differing in their cultural characters as also in their pathogenic behaviour on different gram varieties.

Acknowledgements : My grateful thanks are due to Dr. K. S. Bedi, Ex-Joint Director of Agriculture (R & E), Punjab, Chandigarh and former Plant Pathologist, Punjab, Ludhiana, for suggesting the problem and for valuable guidance throughout the course of these investigations.

Plant Pathological Section,
College of Agriculture,
Punjab Agricultural University,
Ludhiana.

References

Bedi, K. S. (1949) Infectivity and longevity of dried cultures of *Ascochyta rabiei* (Pass.) Lab. the causal fungus of Gram Blight. *Indian Phytopath.*, **2** : 6-10.

Luthra, J. C. and K. S. Bedi (1932) Some preliminary studies on Gram Blight with reference to its cause and mode of perennation. *Indian J. agri. Sci.*, **12** : 499-515.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

LUTHRA, J. C., A. SATTAR and K. S. BEDI (1935) Life-History of Gram Blight [*Ascochyta rabiei* (Pass.) Lab.=*Phyllosticta rabiei* (Pass.) Trot.] on gram and its control in the Punjab. *Agriculture and Livestock in India*, **5** : 489-498.

LUTHRA, J. C., A. SATTAR, and K. S. BEDI (1939) Variation in *Ascochyta rabiei* (Pass.) Lab., the causal fungus of Blight of Gram (*Cicer arietinum* L.). *Indian J. agric. Sci.*, **9** : 791-805.

LUTHRA, J. C., A. SATTAR, and K. S. BEDI (1941) Determination of resistance to Blight disease [*Mycosphaerella rabiei* Kovaceveski= *Ascochyta rabiei* (Pass.) Lab.] in gram types. *Indian J. agric. Sci.*, **11** : 259-264.

LUTHRA, J. C. and A. SATTAR (1941) Control of Gram Blight in the Punjab. *Indian Fmg.*, **2** : 66-69.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ROOT-KNOT OF VEGETABLES. III. FACTORS AFFECTING HATCHING OF EGGS OF MELOIDOGYNE JAVANICA JAVANICA (TREUB, 1885) CHITW., 1949 AND M. INCOGNITA INCOGNITA (KOFOID AND WHITE, 1919) CHITW., 1949*

GOPAL SWARUP AND K. J. PILLAI

(Accepted for publication April 10, 1964)

For any disease to occur in a severe form the presence of abundant active inoculum which is governed by environmental conditions, is essential. In the root-knot disease, incited by the nematodes of the genus *Meloidogyne* Goeldi, 1887, information regarding the various physical and chemical factors affecting the hatching of eggs appears to be meagre. However, preliminary work done with these nematodes and also some other related genera *e.g.*, *Heterodera* has shown that conditions prevailing in the soil including the presence of the chemical stimulants have a marked influence on the hatchability of the eggs. It was, therefore, considered desirable to study the effects of some factors such as temperature, hydrogen-ion concentration, light, aeration and root diffusates on the hatching of eggs of *Meloidogyne* spp., and the results obtained are reported here.

MATERIAL AND METHODS : Single egg mass cultures of the two species, *M. javanica* (Treub, 1885) Chitw., 1949 and *M. incognita* (Kofoid and White, 1919) Chitw., 1949 were started separately in 4″ pots containing sterilized soil and later multiplied in bigger-sized pots, using tomato as the susceptible host plant. As far as possible egg masses uniform in size, age and colour were used in the hatching tests. Single egg masses were individually picked up and kept in glass dishes containing water or other hatching medium.

For collection of root diffusates five 4″ pots having 5 seedlings of each vegetable crop were used. After 3 weeks growth, the pots were filled with water to saturate the soil and then they were filled again with water. The water draining out from the drainage hole at the bottom of the pot was collected in flasks. Fresh root diffusates were collected after every 5 days and used immediately after collection.

EXPERIMENTAL : *Effect of temperature on hatching* : Four egg masses of *M. javanica* and *M. incognita* were put in small dishes containing 2 ml. of tap water. Three such dishes of each were incubated at 8 different temperatures ranging from O°C to 40°C. The hatched larvae were withdrawn at 24 hour intervals and counted. Fresh water was added after each withdrawal of larvae. The results obtained are presented in Tables 1 and 2 and the hatching curve against time is plotted in Figures 1 and 2.

*The 1st paper in this series was published in *Indian Phytopath.*, **15** : 228-230, (1962), and 2nd in *Indian J. Ent.*, **25** : 217-223, (1963).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : The average larval hatch of *M. javanica* at different temperatures

Time in hours	Temperature °C							
	0	5	10	15	20	25	30	40
24	0	4	12	29	134	245	319	53
48	1	3	13	18	114	184	203	34
72	0	1	8	16	83	153	180	39
96	0	2	11	19	82	141	103	29
120	0	1	9	21	51	138	96	18
144	0	4	10	4	53	102	83	16
168	0	1	6	7	43	84	44	17
Total	1	16	69	114	560	1047	1028	206

TABLE 2 : Average larval hatch of *M. incognita* at different temperatures

Time in hours	Temperature °C							
	0	5	10	15	20	25	30	35
24	0	2	8	34	165	325	262	43
48	0	3	10	28	101	244	163	41
72	1	2	16	28	98	186	162	28
96	1	1	8	20	55	176	152	31
120	0	6	9	15	64	104	143	23
144	0	2	11	12	41	86	93	24
168	0	1	13	10	21	53	76	18
Total	2	17	75	147	545	1174	1051	208

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

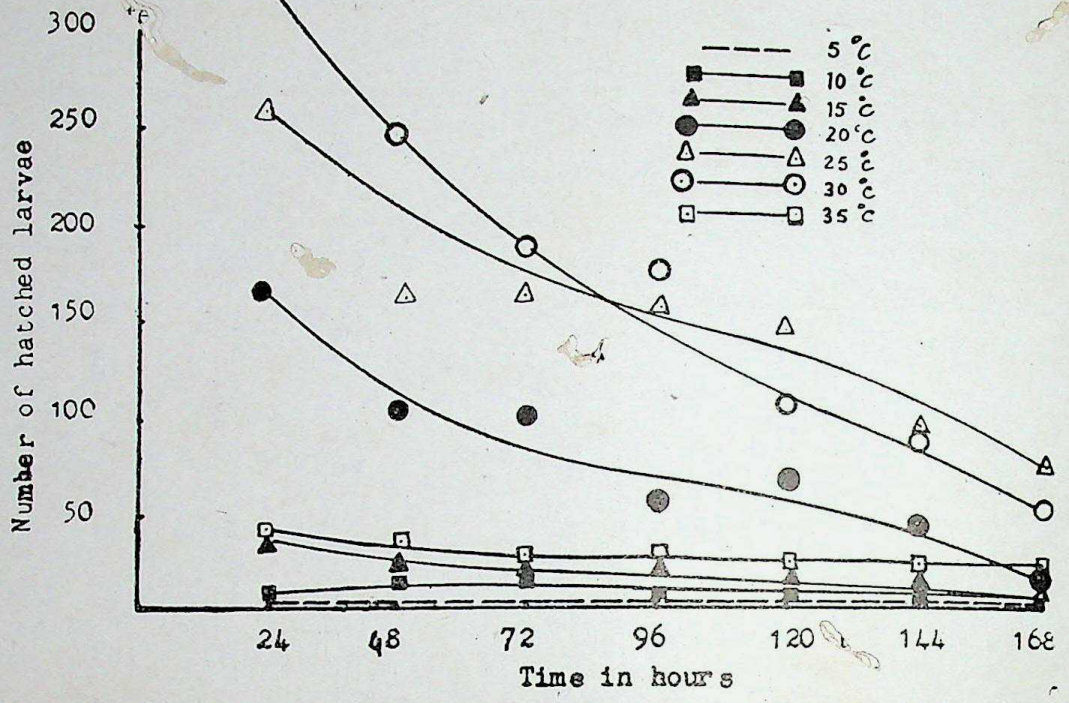


Fig. 1. Effect of temperature on the hatching of eggs of *M. javanica*

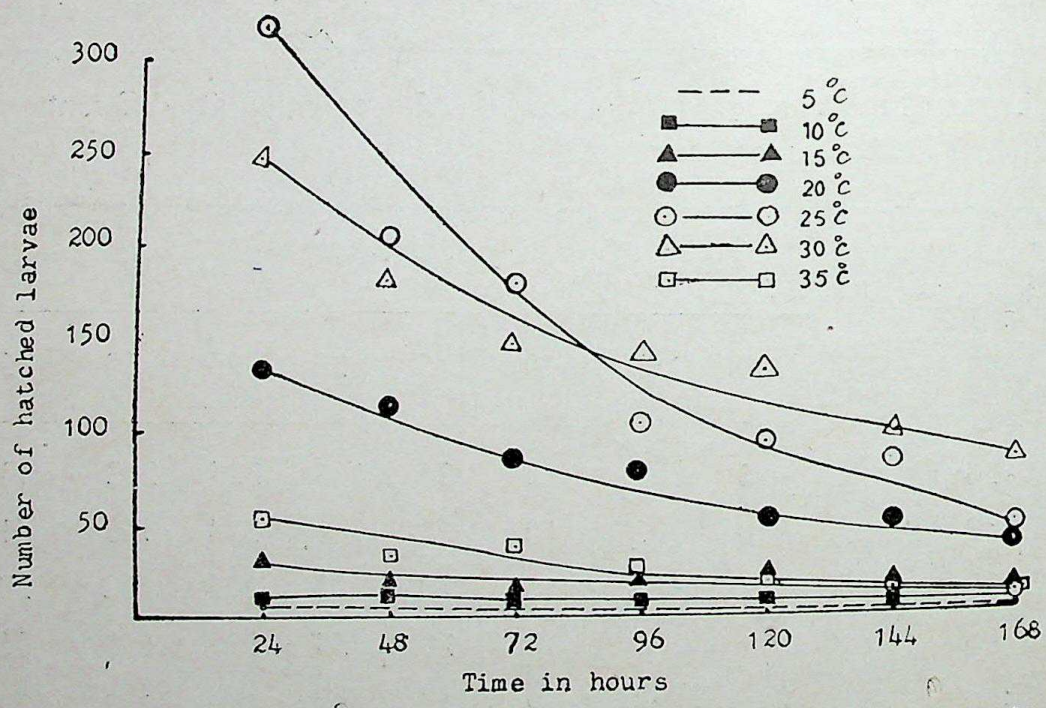


Fig. 2. Effect of temperature on the hatching of eggs of *M. incognita*

Effect of pH on hatching of eggs : Hatching of egg masses at the pH range of 2.0 to 10.0 was studied. Using distilled water as base, pH values from 2.0 to 8.0 were adjusted with the help of citric acid-sodium monohydrogen phosphate buffer solution and pH 9.0 and 10.0 with N/10 sodium hydroxide. The adjusted pH values were checked with

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the Beckman's pH meter, model G. Two egg masses each of the two species under study, were kept separately in small glass dishes containing 2 ml. of the buffered solution. The eggs of *M. javanica* were incubated at 30°C and those of *M. incognita* at 25°C. Three replicates were kept at each pH range. The hatched larvae were withdrawn at intervals of 24 hours and fresh buffered solution was added each day to the glass dishes containing the egg masses. The results are presented in Tables 3 and 4 and the hatching curve against time is plotted in Figures 3 and 4.

TABLE 3 : Average larval hatch of *M. javanica* at different pH concentrations

Time in hours	pH range						
	2.0	4.0	6.0	7.0	8.0	9.0	10.0
24	3	78	136	155	78	9	5
48	0	64	140	148	40	38	0
72	2	86	123	120	34	34	0
96	1	46	98	103	32	29	0
120	0	38	76	76	38	14	0
144	0	29	84	48	29	16	0
168	0	30	61	51	25	21	1
192	0	16	53	39	28	17	0
216	1	17	21	19	26	21	2
240	0	8	18	17	14	8	1
Total	7	412	810	776	344	207	9

TABLE 4 : Average larval hatch of *M. incognita* at different pH concentrations

Time in hours	pH range						
	2.0	4.0	6.0	7.0	8.0	9.0	10.0
24	0	63	140	151	52	24	1
48	3	78	137	130	44	15	0
72	3	72	106	126	38	12	4
96	5	44	89	101	44	12	0
120	0	47	85	98	36	19	2
144	2	34	73	83	39	16	0
168	1	35	50	74	24	13	1
192	0	25	48	68	23	21	0
216	1	22	43	59	14	10	3
240	0	16	24	46	12	12	2
Total	15	436	795	936	326	154	13

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

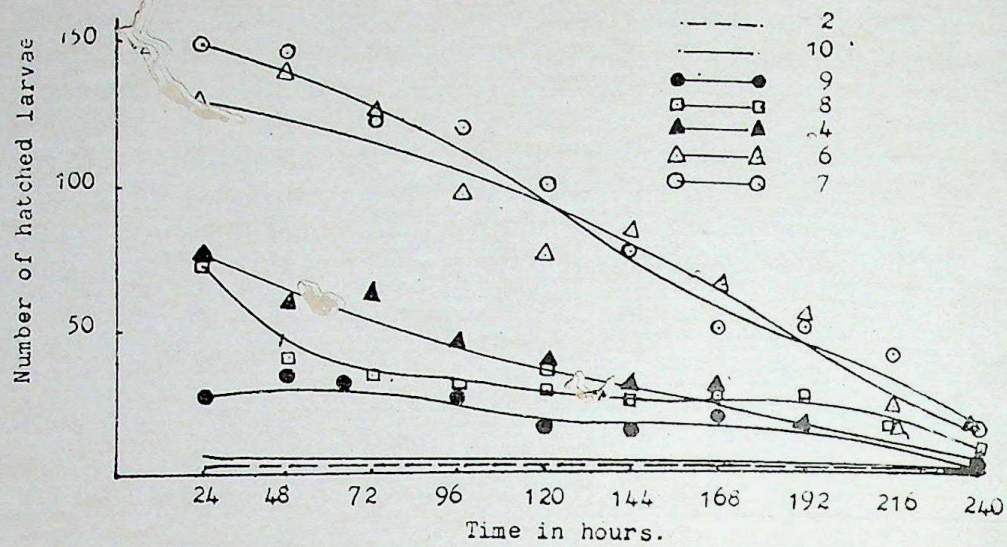


Fig. 3. Effect of hydrogen-ion concentration on the hatching of eggs *M. javanica*

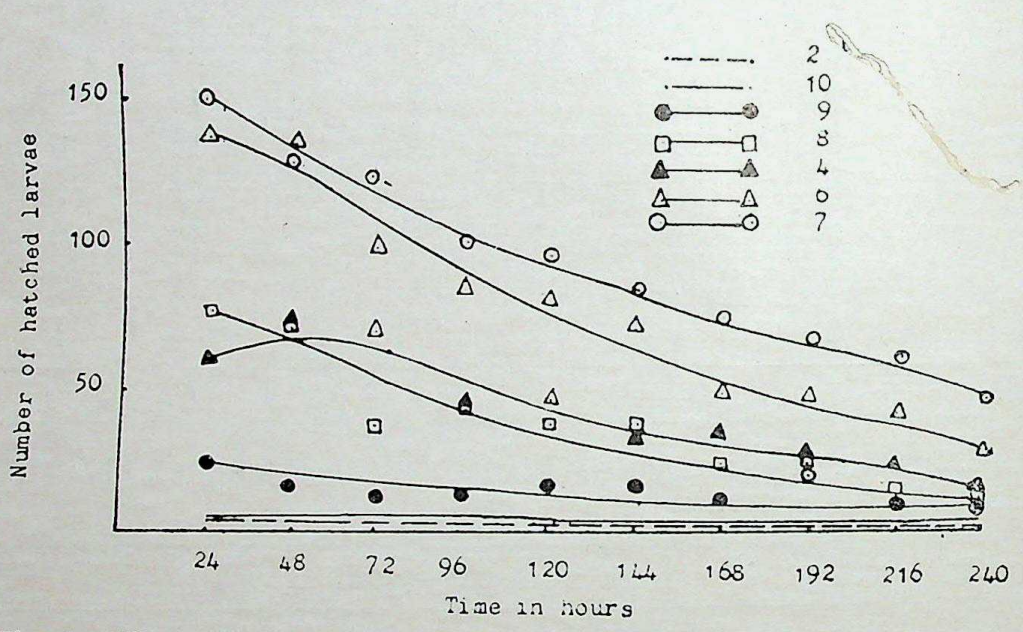


Fig. 4. Effect of hydrogen-ion concentration on the hatching of eggs of *M. incognita*

The response to the hydrogen-ion concentration exhibited by the two species appears to be more or less similar. However, the *javanica* root-knot nematode seems to do equally well at pH 6.0 and pH 7.0 as against the *incognita* nematode for which pH 7.0 was found to be slightly better than pH 6.0.

Effect of light and darkness on the hatching of eggs of M. javanica : Three egg masses, in three replications, were put in glass dishes containing 2 ml. tap water. For continuous light, the dishes were placed in a chamber lighted with a flourescent tube light (15 foot candles), and for continuous darkness, the dishes were wrapped in a thick black paper and opened daily for a very brief period of time needed to suck out the hatched larvae at the end of every 24 hours. Another set of glass dishes were put on laboratory benches where they received alternate

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

light and dark conditions. The results obtained are presented in Table 5 and the hatching curve against time is plotted in Figure 5.

TABLE 5 : Average larval hatch of *M. Javanica* under light and darkness

Interval in hours	Continuous light	Continuous darkness	Alternate light and darkness
24	175	206	162
48	149	180	138
72	112	145	131
96	100	120	116
120	90	102	105
144	78	75	115
168	45	42	83
192	30	38	67
216	13	16	29
Total	792	924	946

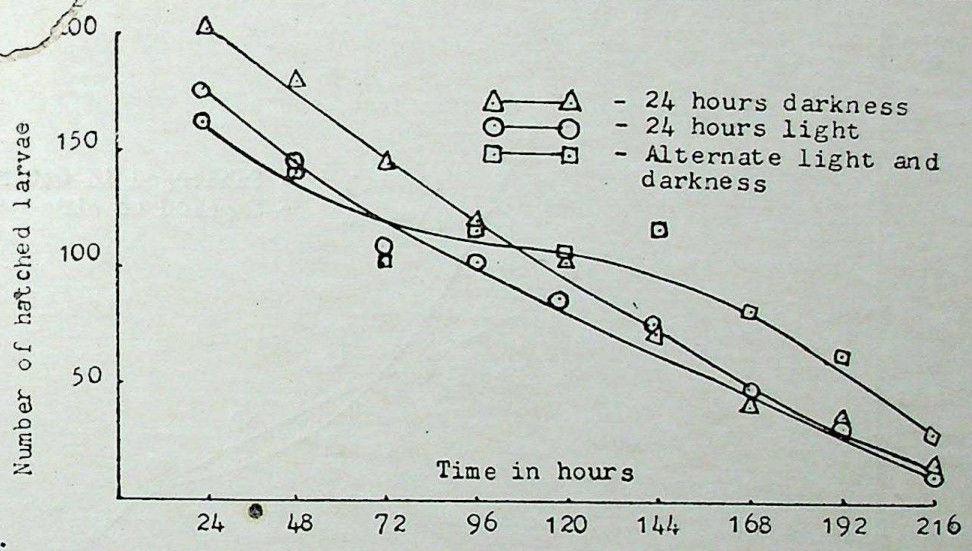


Fig. 5. Effect of light on hatching of eggs of *M. javanica*

It would be observed that there does not appear to be any significant difference in the effect of light or darkness with regard to hatching of eggs. However, there was a slight depression in the hatching under continuous light conditions.

Effect of aeration on the hatching of eggs of M. javanica : Petri-dishes (1-1/2 inch diameter) with a small piece of cloth on top, held in position with the help of a rubber band, were used in this experiment. The cloth-piece, containing 3 egg masses, was pushed just deep enough to touch the water level in the dish. In the other set the egg masses were kept submerged in water. The hatched larvae were pipetted out for counts. The results obtained (average of 4 replications) are presented in Table 6 and the hatching curve against time is plotted in Figure 6.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 6 : Effect of aeration on hatching of eggs of *M. javanica*

Time interval in hours	Average larval hatch of egg masses	
	Free Aeration	Submerged in water
24	288	242
48	289	288
72	207	131
96	151	115
120	127	108
144	98	115
168	78	88
192	63	64
216	24	29
Total	1326	1120

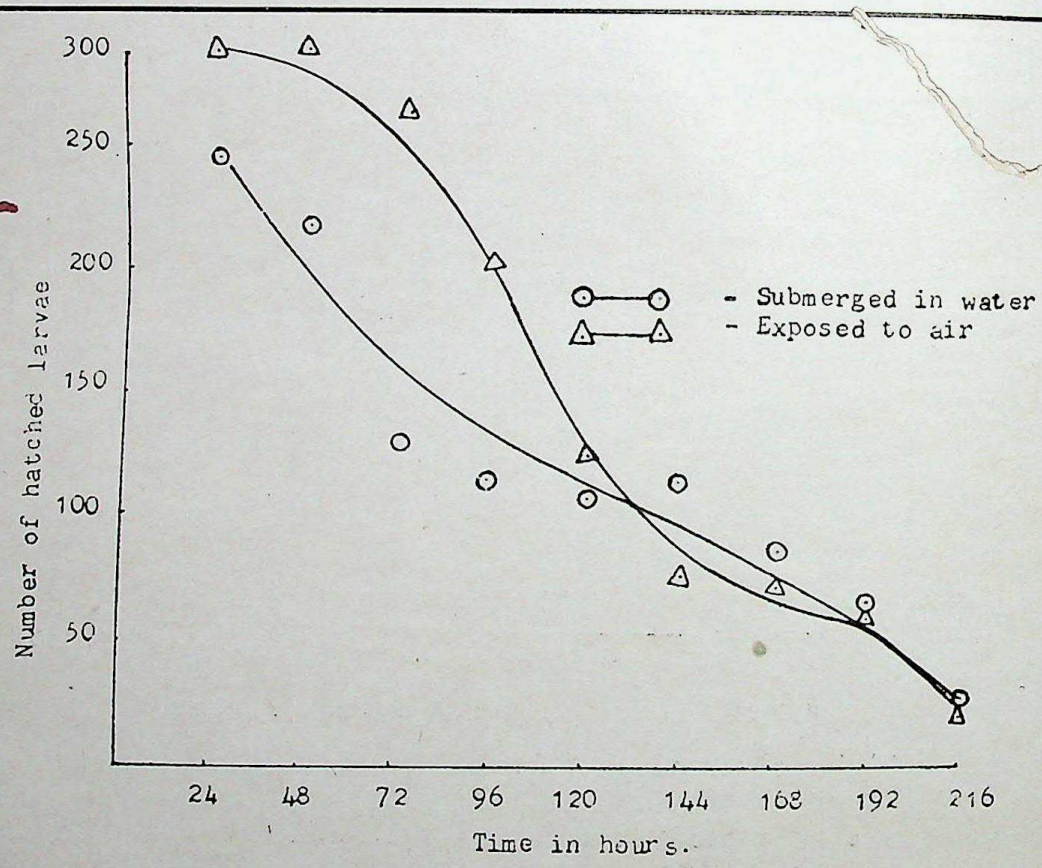


Fig. 6. Effect of aeration on the hatching of eggs of M. *javanica*

After an initial stimulus of free air in increasing the hatching of eggs, there was not much difference between the two different situations in which the egg masses were placed. However, due to this initial increased hatching the total hatch was 18.2 per cent more in free air condition than in the submerged water condition.

Effect of root diffusates on the hatching of eggs of M. javanica : Root diffusates being an important factor in hatching of eggs of some species of *Heterodera* provided a stimulus for similar work in this case

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

of root-knot nematodes where, so far, very little attention has been paid presumably because of the fact that a good hatch is always obtainable in water. Recently, Viglierchio and Lownsberry[3] reported 15-30 per cent increase in hatching of eggs of *M. hapla*, *M. javanica* and *M. incognita* using tomato root emanations. In the present experiment, root exudates from 7 different crops namely tomato (*Lycopersicon esculentum*), *bhindi* (*Abelmoschus esculentus*), radish (*Raphanus sativus*), cabbage (*Brassica oleracea* var. *capitata*), cowpea (*Vigna sinensis*), groundnut (*Arachis hypogea*) and pea (*Pisum sativum*) were tried. Four egg masses were kept in each of the Petri-dishes (1½ inches diameter) containing 2 ml. of the root diffusate. Egg masses in water served as control. The dishes were incubated at 30°C., the hatched larvae being withdrawn and counted every 24 hours. The results (average of 3 replications) are presented in Table 7 and the hatching curve against time is plotted out in Figure 7.

TABLE 7 : Effect of root diffusates of different crops on the hatching of eggs of *M. javanica*

Time interval in hours	Number of larvae hatched in root diffusates of							
	Tomato	*Bhindi*	Radish	Cabbage	Cowpea	Ground-nut	Pea	Water
24	388	196	277	282	162	371	146	172
48	263	143	214	212	152	264	175	184
78	186	176	179	181	187	210	102	163
96	180	162	149	148	89	164	84	161
120	188	150	146	163	95	119	81	101
144	123	96	55	95	85	74	82	82
168	90	80	39	46	49	62	83	93
192	93	40	27	28	29	35	43	67
216	43	38	20	25	16	31	25	32
Total	1554**	1081	1106	1180	864	1330*	821	1055

**C.D. at 1 per cent level, 314.4 ; *at 5 per cent 212.6.

It would be observed that tomato and groundnut root diffusates were very effective in inducing a greater hatch than water. Taking water hatch as the base, tomato and groundnut root diffusates gave 47.2 and 26.0 per cent more hatch, respectively over water while the root diffusates from pea and cowpea depressed the hatch by 22.1 and 18.1 per cent, respectively. After the initial flux of larvae in the case of tomato and groundnut root diffusates, a drop in the hatching rate was evident whereas in all the other cases the hatching rate remained more or less uniform (Fig. 7).

DISCUSSION : The importance of various physical and chemical factors in hatching of eggs can not be over emphasised. Prior to 1949, work

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

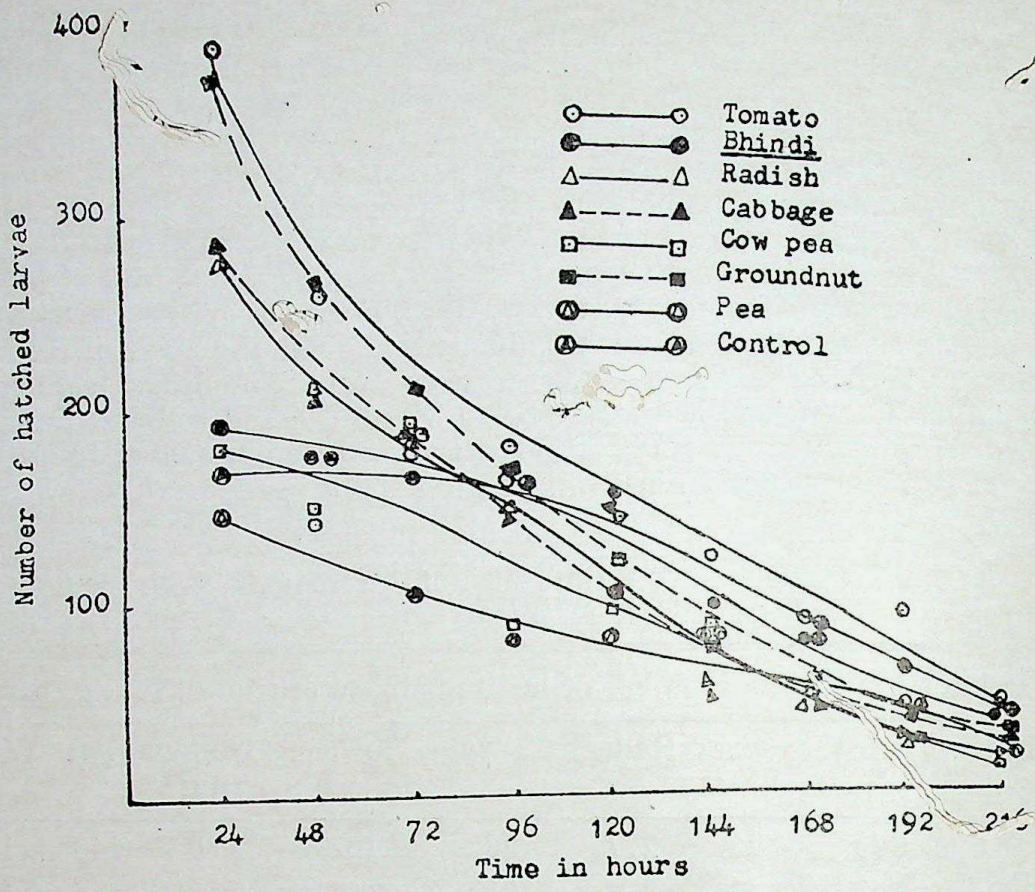


Fig. 7. Effect of different root diffusates on the hatching of eggs of *M. javanica*

done by Godfrey[2] on *Heterodera marioni* had shown that the most favourable temperature for hatching was between 24 to 28°C. With the present distinction of species in the root-knot nematodes, this information became obsolete. The present studies indicate that though both the species, *M. incognita* and *M. javanica*, are different morphologically yet they behave as one unit at least with regard to temperature and pH requirement in hatching of eggs. From an ecological view point this information is important in assessing probable distribution of these nematodes within the country. The fact that both these species can tolerate a wide range of temperature and hydrogen-ion concentration, accounts for the reports from various part of the country about the occurrence of these two species of *Meloidogyne*.

The effect of root diffusates on the hatching of eggs has been definitely shown in the case of *M. javanica*. Besides tomato, which definitely increased the hatching, groundnut also significantly influenced the hatching. On the other hand pea root diffusate had a marked depressing effect. It is interesting to observe that groundnut, which is a non-host, for this species, has a stimulatory influence on the hatching of eggs. Such an observation has been recorded for *Heterodera* spp. by Fenwick[1], but there does not appear to be any such record for *M. javanica*.

SUMMARY

The best temperature and pH for hatching of eggs of both *M. javanica* and *M. incognita* was found to be between 25-30°C and pH 6 to 7

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

respectively. Continuous light slightly depressed hatching of eggs of *M. javanica*.

There was no marked difference in the total number of larvae hatched when eggs were kept submerged in water or under free oxygen condition.

Tomato root diffusate significantly improved hatching of *M. javanica* eggs.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. R. S. Vasudeva and Dr. B.L. Chenulu former and the present Head of the Division of Mycology and Plant Pathology. for their keen interest and helpful criticism and for going through the manuscript critically.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

[1]Fenwick, D. W. *J. Helminth.*, **24** : 75-86 (1950).
[2]Godfrey, G.H., *Phytopathology*, **21** : 323-329 (1931).
[3]Viglierchio, D.R. and Lownsberry B.F. *Nematologica*, **5** : 153-157 (1960).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FACTORS INFLUENCING LARVAL HATCHING IN THE ROOT-KNOT NEMATODES, MELOIDOGYNE INCOGNITA (KOFOID AND WHITE, 1919), CHITWOOD, 1949. AND M. JAVANICA (TREUB, 1885), CHITWOOD, 1949.

I—EFFECT OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION

ASAD AHMED AND ABRAR M. KHAN

(Accepted for publication March 17, 1964)

Calam, Raistrick and Todd (1949), Lownsbury (1950, 1951) and Fenwick (1951) studied the effect of temperature on larval hatching in *Heterodera rostochiensis* Wollenweber. Similarly, Wallace (1955) and Winslow (1955) studied the effect of temperature on *H. schachtii* Schmidt. and *H. avenae* O'Schmidt respectively. The effect of hydrogen-ion concentration on hatching in *H. rostochiensis* has been studied by Ellenby (1946), Calam, Todd and Waring (1949), Fenwick (1951) and Robinson and Neal (1956). As little work has been done on larval hatching in the root-knot nematode, *Meloidogyne* spp., the effect of temperature and hydrogen-ion concentration was studied on *M. incognita* (Kofoid and White, 1919), Chitwood, 1949, and *M. javanica* (Treub, 1885), Chitwood, 1949.

MATERIAL AND METHODS : Mature females of *M. incognita* and *M. javanica* were obtained from infested roots of *Abelmoschus esculentus* Moench. (*Okra*) and *Eranthemum* sp. respectively. Ahmed (unpublished data) has previously shown that hatching is stimulated by okra root leachate. Hence okra root leachate was employed as the hatching medium, besides distilled water. Okra seedlings were germinated on moist quartz sand in petri-plates and transferred to glass troughs containing sterilised quartz sand. Leachates were obtained from one-week old seedlings by pouring distilled water into trough and drawing off the root diffusates by pipette after four hours.

To study the effect of temperature, batches of twenty mature females were dissected from infested okra roots and were later placed in 18 small petri-plates, half of which contained 10 ml. of the leachate, and the remaining half 10 ml. of distilled water, per plate. The plates were then incubated at 0°, 10°, 15°, 25°, 30°, 35°, 40° and 50°C for seven days. At the end of the period, the hatching fluid and the hatched larvae were drawn off by a pipette and the number of larvae emerged was counted on a Hawksley counting slide.

For studying the effect of hydrogen-ion concentration, distilled water and okra leachate were adjusted to the different pH values within the range of 2.0-10.5 (Robinson and Neal, 1956). Batches of twenty adult females were tested in 10 ml. of different hatching media. Assays were run in triplicates for a week at 25°C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL RESULTS : *Effect of temperature on hatching* : The effect of temperature on hatching of larvae of *Meloidogyne incognita* and *M. javanica* is shown in Fig. 1. There was no hatching at 0°C and 50°C in distilled water in both the species though poor hatching occurred in leachates at these temperatures. Optimum temperature for hatching of *M. incognita* eggs was 25°C and 30°C for *M. javanica* eggs, both in water as well as in leachates. These findings are in conformity with

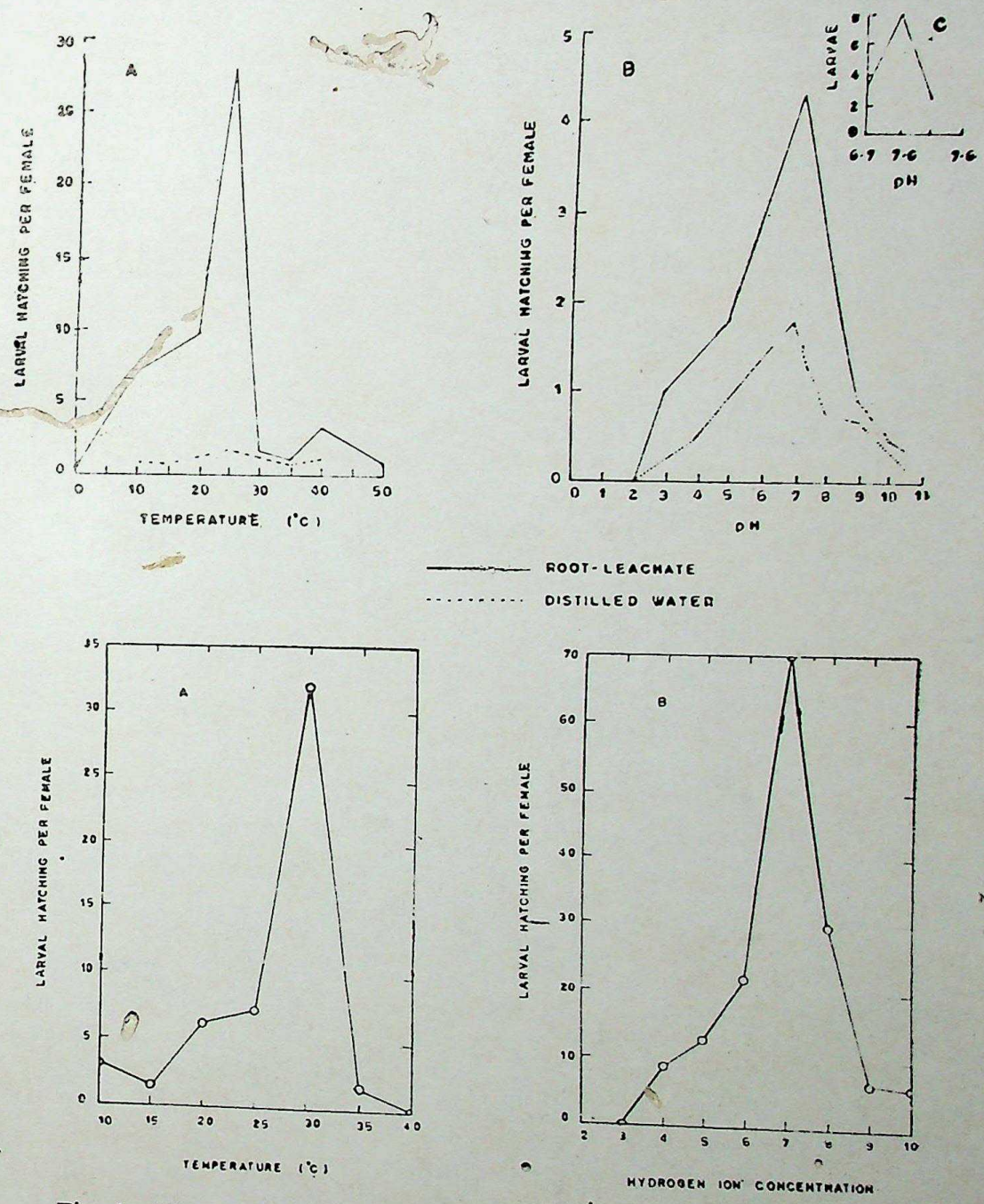


Fig. 1. The influence of temperature and Hydrogen-ion concentration on larval hatching in the root-knot nematode (I) *Meloidogyne incognita* and (II) *M. javanica* in distilled water and okra seedlings.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the results obtained by Thomson (1957) that reproduction in *M. javanica* becomes inconsistent and reduced markedly at 35°C.

Effect of pH on hatching : The influence of pH on larval hatching is presented in Fig. 1 (Band B'). In the case of *M. incognita* hatching was inhibited, both in distilled water as well as in leachates at pH 2.0. Hatching started at pH 3.0 and increased steadily with increase in the pH of the medium, the maximum hatch being obtained at pH 7.0. With further increase in pH the hatching diminished gradually till little hatching was observed at pH 10.5.

To investigate the optimum pH for hatching more precisely another experiment was conducted in which the pH of the leachate was adjusted in a narrow range *viz.*, 6.7, 7.0, 7.3 and 7.6. Larval counts after a week showed that maximum hatching occurred at pH 7.0.

In the case of *M. javanica* no hatching occurred at pH 2.0 and 3.0. However, hatching was initiated at pH 4.0 with maximum hatching occurring at pH 7.0.

DISCUSSION : Results of the experiment on temperature indicate that hatching of the larvae in *M. incognita* and *M. javanica* occurs at a wide range of temperatures. These results also agree with those of Thomson (1957) who reported that reproduction in *M. incognita acrita*, *M. hapla*, and *M. javanica* is arrested at 35°C.

Fig. 1-A shows that the effect of temperature on larval emergence is not so marked as on the leachate potency. The differences in larval hatching at 20°, 25° and 30°C in distilled water and in the leachate can not be attributed solely to hatching stimulation by the leachate and hence, it has been attributed to leachate potency. This enhanced potency might be due to the optimum activity of the factor (or factors) at 25°C and 35°C which might be less effective at other temperatures. A similar abrupt rise, followed by a rapid fall has been reported by Calam *et al.*, (1949) for the potato-eelworm hatching factor.

The hydrogen-ion concentration of the medium had a pronounced effect on larval hatching in *M. incognita* and *M. javanica* but the optimum pH for both the species is 7.0. The optimum for these species differs from the values reported by Calam *et al.*, (1949) and Robinson and Neal (1956) for the potato root-eelworm.

ACKNOWLEDGEMENTS : The authors wish to express their thanks to Mr. F. G. W. Jones, Head, Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts., U.K., and Dr. S. K. Saxena, Department of Botany, Aligarh Muslim University, Aligarh for offering helpful suggestions.

Botany Department,
Aligarh Muslim University,
Aligarh (U.P.)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

CALAM, C. T., H. R. RAISTRICK, AND A. R. TODD, (1949) The potato-eelworm hatching factor. 1. The purification of concentrates of the hatching factor and a method of bioassay. *Biochem. J.*, **45** : 513-519.

CALAM, C. T., A. R. TODD, AND W. S. WARING, (1949) The potato-eelworm hatching factor. 2. Purification of the factor by alkaloid salt fractionation. Anhydrotetronic acid as an artificial hatching agent. *Biochem. J.*, **45** : 520-524.

ELLENBY, C. (1946) Ecology of the eelworm cyst. *Nature, Lond.*, **157** : 451-452.

FENWICK, D. W.(1951) Investigations of the emergence of larvae from the cysts of the potato root-eelworm, *Heterodera rostochiensis*. 4. Physical conditions and their influence on larval emergence in the laboratory. *J. Helminth.*, **25** : 37-48.

LOWNSBURY, B. F. (1950) Stimulation of the Golden nematode larvae by root leachings. *Phytopathology*, **40** : 18 (Abst.).

———————(1951), Larval emigration from cysts of the Golden Nematode of potatoes, *Heterodera rostochiensis* Wollenweber. *Phytopathology*, **41** : 889-896.

RHODE, R. A. AND W. R. JENKINS (1957) Effect of temperature on the life-cycle of stubby root-nematodes. *Phytopathology*, **47** : 29 (Abst.).

ROBINSON, T. AND A. L. NEAL, (1956) The influence of hydrogen-ion concentration on the emergence of Golden Nematode larvae. *Phytopathology*, **46** : 665-667.

THOMSON, I. J. (1957) Influence of soil temperature on reproduction of *Meloidogyne* spp. *Phytopathology*, **47** : 34-35 (Abst.).

WALLACE, H. R. (1955) Factors influencing the emergence of larvae from cysts of bean eelworm, *Heterodera schachtii* Schmidt. *J. Helminth.*, **29** : 3-16.

WINSLOW, R. D. (1955) The hatching responses of some root-eelworms of the genus *Heterodera*. *Ann. appl. Biol.*, **43** : 19-36.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FACTORS INFLUENCING LARVAL HATCHING IN THE ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA (KOFOID AND WHITE, 1919), CHITWOOD, 1949

II—EFFECT OF ROOT LEACHATES AND CERTAIN CHEMICALS

ASAD AHMED AND ABRAR M. KHAN

(Accepted for publication March 17, 1964)

Some parasitic nematodes respond to minute quantities of substances liberated by host roots. This was recognised by Baunacke (1922) who found that larval hatching increased considerably when the cysts of *Heterodera schachtii* Schmidt (Sugar-beet nematode) were placed in water which had been in contact with the roots of host plants. Triffitt (1930), O'Brien and Prentice (1930), Calam, Todd and Waring (1949) and Fenwick (1949) have studied hatching stimulation and the hatching factors in *H. rostochiensis* Wollenweber. Winslow (1955) described the hatching responses of nine species of *Heterodera* and found, with some exceptions, that they responded to root-exudates from the host plants. Winslow and Ludwig (1957) investigated the hatching stimulation of *Heterodera schachtii* with beet and rape root diffusates. Doncaster (1956) and Hesling (1957) have also studied the hatching responses of *H. cruciferae* Franklin and *H. avenae* O'Schmidt respectively.

Peacock (1957) stated that hatching in the root-knot nematode (*Meloidogyne* spp.) was independent of the influence of root-diffusates. However, preliminary experiments by the authors indicated that root-diffusates stimulated larval hatching in *M. incognita* (Kofoid and White, 1919) Chitwood, 1949. Attempts were, therefore, made to determine the activity of root-diffusates of some host and non-host plants, to study the production of hatching stimulants from various regions of okra and tomato seedlings; to study the effect of duration of leach, seedling number and age of seedlings on leachate potency and to test for the efficacy of various pure chemicals as hatching stimulants.

MATERIAL AND METHODS : Root leachates of four host plants *viz.*, bottle gourd (*Lagenaria vulgaris* Ser.), okra (*Abelmoschus esculentus* Moench.), *Solanum nigrum* L. and tomato (*Lycopersicon esculentum* L.) and three non-host plants *viz.*, *Sorghum vulgare* Pers., *Triticum aestivum* L. and Bajra (*Pennisetum typhoideum* Rich.) were tested. The seedlings were grown in sterilised quartz and the leachates were prepared by immersing the excised roots of 4-day old seedlings in 10 ml. of distilled water in petri-plates for four hours.

To test the production of hatching stimulants by different regions of plant seedlings, okra and tomato seeds were germinated in sterilised quartz. When the seedlings were three days old, several 6 mm. pieces were excised from the tomato roots. Leachings were prepared by immersing separately the root pieces of different regions in 10 ml. of distilled water in petri-plates for four hours.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In testing the effect of duration of leach, seedling number and age of seedlings on leachate potency, the roots of 20 okra seedlings were immersed in 100 ml. of distilled water. Samples of leachate were collected after every four hours, and their potency tested. Similarly, leachates obtained by immersing different numbers (*viz.*, 2, 4, 8 and 10) of germinating okra seedlings in 20 ml. of distilled water were used for testing the influence of seedling concentration. The effect of age of seedlings was tested by preparing leachates of okra seedlings, varying in age from 1 to 7 weeks in 10 ml. of distilled water for 2 hours. The seedlings were removed and the leachates were tested for their potency.

To test the effect of carbohydrates, amino acids, vitamins and hormones, aqueous solutions were prepared of the strength of 10^{-2} M. Starch was used at the rate of 0.5 per cent solution in water. A drop of toluene was added to each of the petri-plates containing 10 ml. of the aqueous solution of the various chemicals tested.

In all the above studies 10 ml. of the aliquot contained in petri-plates was inoculated with 20 mature females of *Meloidogyne incognita* taken out of the heavily infested roots of okra. Hatching of eggs in 10 ml. of distilled water served as control. Each experiment was replicated 3 to 5 times; thus the number of females tested in each case ranged from 60 to 100. Plates were incubated for a week at 25°C after which the emerged larvae were counted.

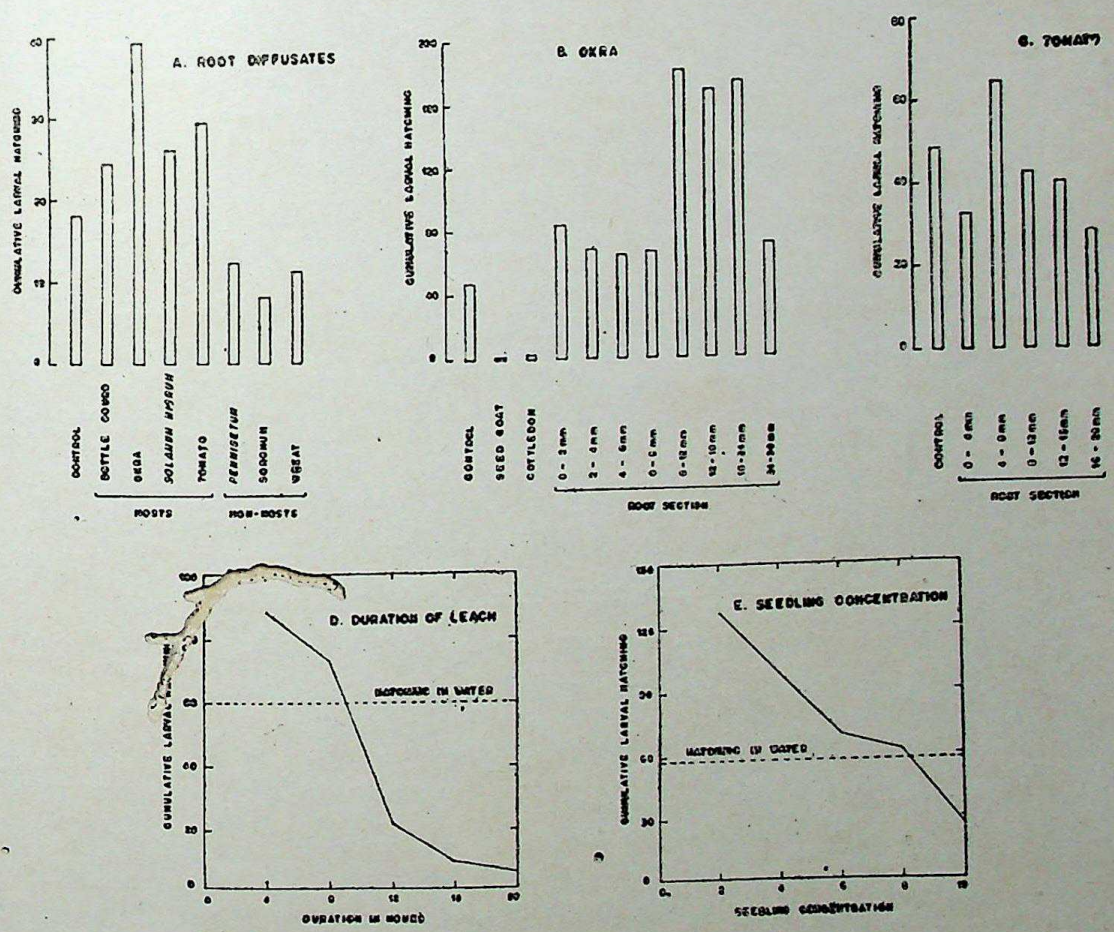


Fig. 1. Hatching stimulation of the root-knot nematode, *Meloidogyne incognita*. (a) Larval hatching in host and non-host root diffusates. The influence of different regions of okra (b) and tomato (c) seedlings, duration of leach (d) and seedling concentration (e) on leachate potency.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL RESULTS : It is clear from Fig. 1 (A) that wide variations exist in the hatching activity of host and non-host root diffusates. The host root diffusates tend to stimulate the hatching.

The seed-coat and cotyledons (Fig. 1 B) of okra appear to have inhibitory effect as hatching was suppressed in these leachates. However, the different regions of okra roots stimulated hatching to varying extent. The regions 0-6 mm., and 24-30 mm. from the root tip appeared to be relatively less stimulatory than the middle portion *i.e.*, 8-24 mm. from the tip. Fig. 1 C shows the hatching activity of different regions of tomato roots. The leachate from 0-4 mm. piece from the tip gave a smallar hatch than in distilled water. Highest hatching occurred in the region 4-8 mm. from the tip but the activity gradually decreased in subsequent segments. The optimum hatching activity was shown by leachate collected after 4 hours (Fig. 1 D). However, as the duration of leach increased the hatching activity decreased. The effect of seedling number was similar (Fig. 1 E). The highest hatch occurred in the leachate prepared by immersing two seedlings and leachate potency decreased as the number of seedlings increased.

The effect of age of seedlings on leachate potency is shown in Fig. 2. Larval hatching was poor in distilled water as well as in the leachate obtained from ungerminated seeds. However, hatching was stimulated in germinated seedlings and increased steadily with increase in the age of seedlings.

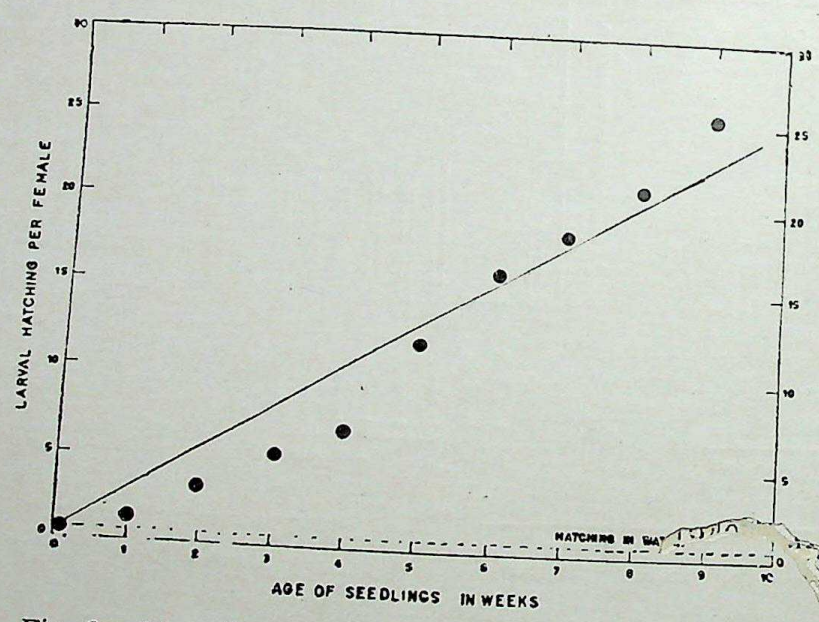


Fig. 2. The effect of age of okra seedlings on leachate potency for larval hatching in the root-knot nematode, *Meloidogyne incognita*.

Effect of different carbohydrates on hatching : Marked variations were observed in the influence of different carbohydrates on larval hatching in *M. incognita* (Fig. 3.). Of the twenty two carbohydrates tested, fourteen increased the rate of hatch. Among these were l(+) arabinose, d (+) dextrose, dulcitol, d (+) galactose, d (+) mannitol, d (+) melibiose, l (+) rhamnose, d (—) ribose, d (—) sorbitol, d (+) sucrose and d

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(+) xylose. Larval hatching was highest in l (+) arabinose and d (—) sorbitol. Aqueous solutions of d (+) maltose, soluble starch and l (—) sorbose had little or no effect compared with distilled water. Other carbohydrates *viz.*, adonitol, l (—) arabitol, d (+) cellibiose, d (+) mannose and d (+) raffinose had an inhibitory effect and hatching was completely suppressed in solutions of d (+) mannose and d (+) raffinose.

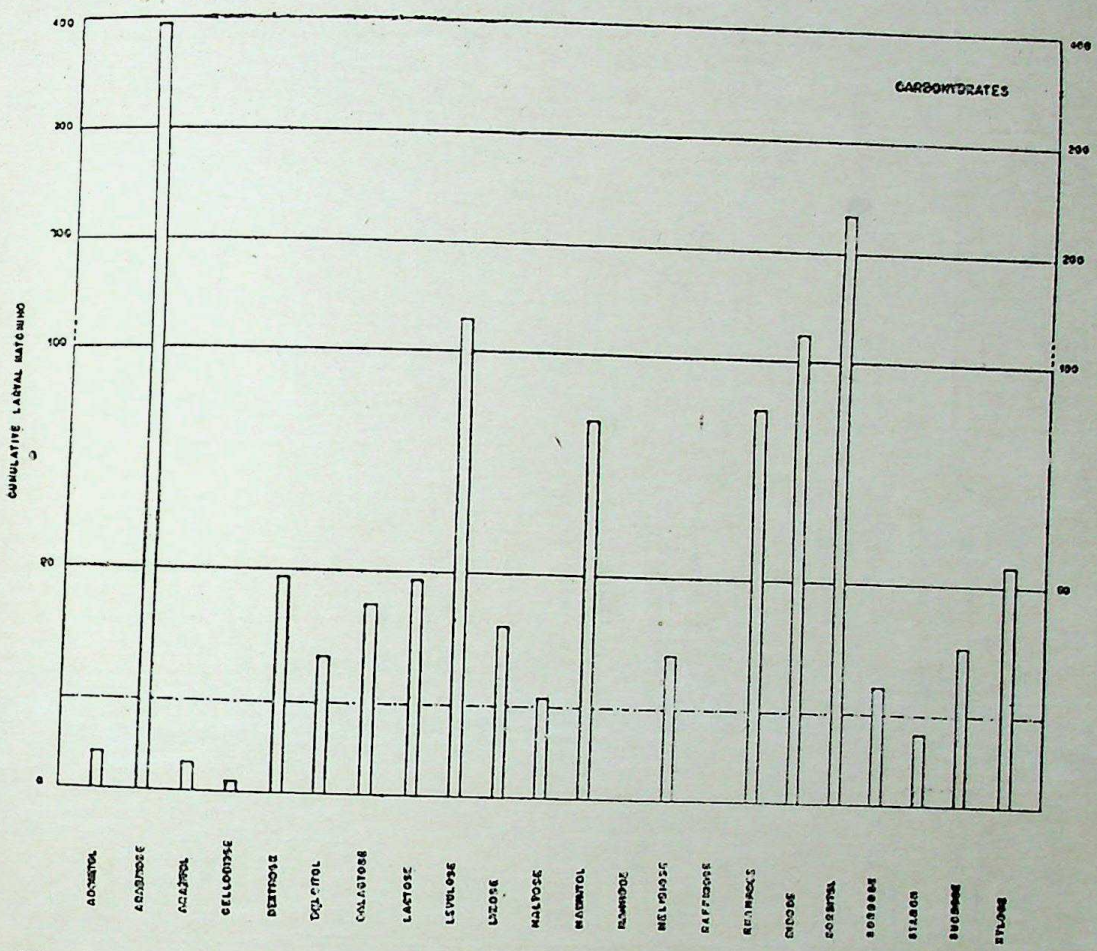


Fig. 3. Hatching of the root-knot nematode, *Meloidogyne incognita* in different carbohydrate solutions. The horizontal dotted line indicates the level of larval hatching in distilled water.

Effect of different amino-acids on hatching : Fig. 4 shows that aqueous solutions of L-glutamic acid, L-histidine, L-leucine, L-lysine hydrochloride and DL-methionine were distinctly stimulatory as larval hatching in these amino acids was higher than that observed in water. The cumulative hatch was highest in L-leucine. But the majority of the amino-acids tested *viz.*, DL alpha alanine, L-arginine, L-asparagine, creatine, glycine, DL-tryptophane and DL-valine exhibited inhibitory effect on hatching. The cumulative hatch was greatly reduced in DL-alpha alanine, L-asparagine and DL-valine. L-cysteine had little or no effect as the rate of hatching closely approximated to that observed in distilled water.

Effect of vitamins and growth promoting substances on hatching : Hatching was very poor in most of the vitamins tested (Fig. 5). All the vitamins excepting ascorbic acid and biotin suppressed larval hatch to

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

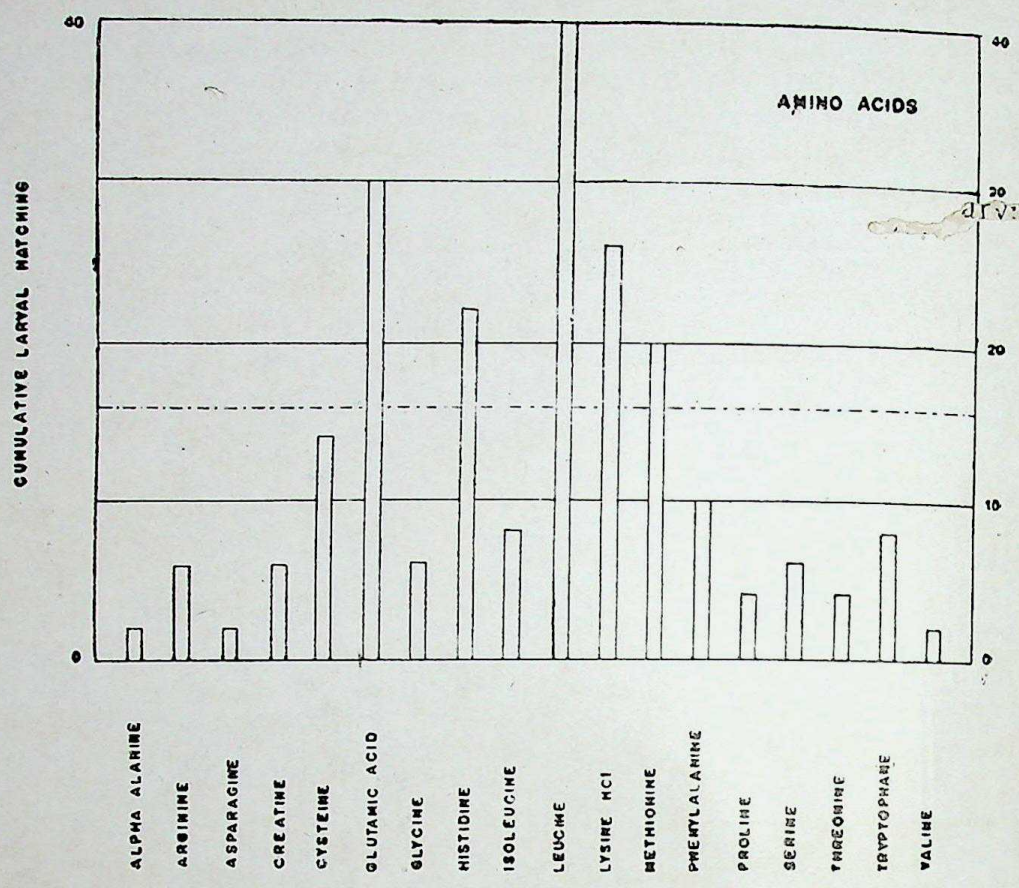


Fig. 4. Hatching of the root-knot nematode, *Meloidogyne incognita* in different amino-acid solutions. The horizontal dotted line indicates the level of larval hatching in distilled water.

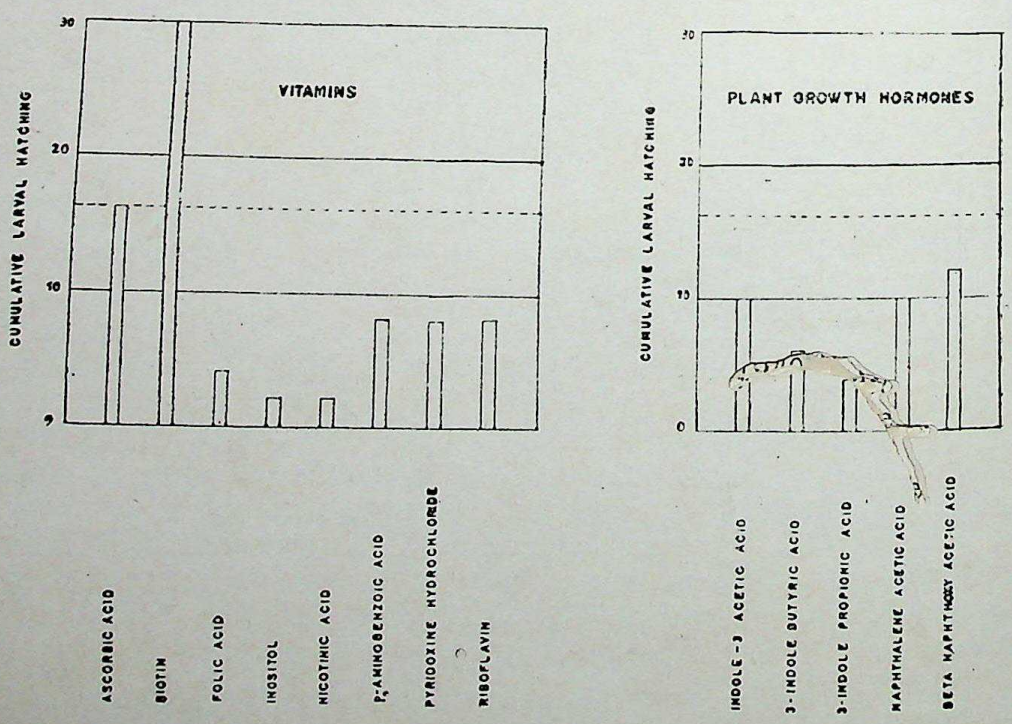


Fig. 5. Hatching of the root-knot nematode, *Meloidogyne incognita* in solutions of different vitamins and plant growth hormones. The horizontal dotted line indicates the level of larval hatching in distilled water.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

a marked extent. However, ascorbic acid was almost neutral in its effect and biotin stimulated hatching.

Plant growth substances also suppressed hatching of the larvae (Fig. 5).

DISCUSSION : The results show that hatching in the root knot nematode, *Meloidogyne incognita* is stimulated by root-diffusates produced by host plants. Highly potent leachates are produced by okra, tomato, bottle gourd and *Solanum nigrum* roots, whereas wheat, sorghum and *Pennisetum typhoideum* root-leachates have little stimulatory activity.

The stimulatory substances are produced chiefly by the roots of the seedlings and not from the seed-coat or cotyledons. These results confirm the findings of Winslow and Ludwig (1957) for beet eelworm. The region of 0 to 6 mm. from the tip of the okra roots which comprises of the root cap, apical meristem and the zone of elongation is less stimulatory than the 6-24 mm. root segment which includes the region of maturation and root-hairs. The 24-30 mm. region which consists of the hypocotyl does not produce potent leachates. Similar results were obtained in case of tomato roots. Optimum hatching activity is shown by leachates obtained from 4-8 mm. region which again consists of the region of maturation. However, the stimulatory activity gradually decreased in the region behind 8 mm. and little activity was shown by the 16-20 mm. region. The potency of the leachates gradually decreased with increase in duration of leach. Similarly, the hatching activity of the leachate decreased with an increase in the seedling concentration. This gradual decrease in potency may be due to an increase in the osmotic pressure of the leachate (unpublished data), a factor which has been emphasised by Wallace (1955).

An increase in the age of the seedlings appears to have a favourable effect on leachate potency. The hatching activity of 9-week old okra seedlings was about five times that of three week old seedlings. These observations are in conformity with the reports of Winslow (1955) and Winslow and Ludwig (1957). This increased potency may be explained by assuming greater accumulation of the hatching factor in older seedlings or it may also be due to the large surface area exhibited by the older seedling-roots, which permits liberation of the factor in larger quantities.

The results on the effect of carbohydrates on hatching of larvae indicate that carbohydrates as a whole, are effective hatching stimulants for *M. incognita*. Most carbohydrates tested gave increase in the rate of hatching while only a few were inhibitory. A relationship can be traced between the chemical configuration of these substances and their activity as hatching stimulants. The mean cumulative hatch in the various pentoses tested was 140, on the other hand, in hexoses it was 49 larvae. The disaccharides are poor stimulants as the average cumulative hatch was 28 larvae. Finally, no hatch occurred in starch, a polysaccharide.

Amino acids are less stimulatory to hatching than the carbohydrates as none of the eighteen amino acids tested gave as much hatching as was observed for most of the carbohydrates. Excepting leucine all

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

monoamino monocarboxylic acids showed poor stimulation or suppressed hatching. However, glutamic acid, the only monoamino dicarboxylic acid included in these experiments, displayed a favourable effect. On the other hand, the basic amino acids differed widely in their influence as arginine and creatine suppressed hatching whereas lysine hydrochloride and histidine appeared to favour it. The sulphur containing aminoacids *viz.*, cysteine and methionine behaved as almost neutral as the rate of hatching was more or less the same as observed in distilled water. Asparagine, an amide of mono-amino-dicarboxylic acid, exhibited distinct inhibitory effect on hatching. Vitamins in general did activate hatching in *Meloidogyne incognita*.

The plant growth hormones also displayed pronounced suppression of hatching in *M. incognita*. This seems to rule out the possibility that the growth hormones produced by the apical regions of the roots are responsible for stimulation of larval hatching.

Summary

The influence of several host and non-host root leachates and some carbohydrates, amino acids, vitamins and plant growth substances on hatchihg of the larvae of the root-knot nematode, *Meloidogyne incognita* has been studied. Root-diffusates from host plants *viz.*, bottle gourd, okra, tomato and *Solanum nigrum* gave favourable responses whereas leachates of non-host *viz.*, wheat, sorghum and *Pennisetum typhoideum* did not stimulate hatching of larvae. Highest stimulatory activity was shown by leachings obtained from the region of maturation of the young roots.

Most of the carbohydrates tested increased the rate of hatching. Amino acids were less stimulatory to hatching than the carbohydrates. Vitamins in general did not activate hatching whereas plant growth hormones suppressed the hatching of the larvae of *M. incognita*.

Acknowledgements : The authors wish to thank Mr. F. G. W. Jones, Head of the Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts, U.K., and Dr. S. K. Saxena, Department of Botany, Muslim University, Aligarh for going through the manuscript and offering helpful suggestions.

Botany Department,
Aligarh Muslim University,
Aligarh. (U.P.)

References

Baunacke, W. (1922) Untersuchungen zur Biologie und Bekaempfung des Rueben-nematoden, *Heterodera schachtii* Schmidt. *Arb. biol. Reichasanslt Land u. Forstwirtsch.* **11** : 185-288.

Doncaster, C. C. (1956) Some observations on the hatching responses of the cabbage root eelworm, *Heterodera cruciferae* Franklin. *Ann. appl. Biol.*, **44** : 283-291.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FENWICK, D. W. (1949) Investigations on the emergence of larvae from cysts of the potato-root eelworm, *Heterodera rostochiensis*. I-Technique and variability. *J. Helminth.*, **23** : 157-170.

HESLING, J. J. (1957) The hatching responses of *Heterodera major* O'Schmidt. to certain root-diffusates. *Nematologica*, **2** : 123-125.

O'BRIEN, D. G. and E. G. PRENTICE (1930) An eelworm disease of potatoes caused by *Heterodera schachtii*. *Scot. J. Agric.*, **13** : 415.

PEACOOK, F. C. (1957) Plant Parasitic eelworms. *Outlook Agric.*, **1** : 188-196.

TRIFFITT, M. J. (1930) On the bionomics of *Heterodera schachtii* on potatoes with special reference to the influence of mustard on the escape of larvae from the cysts. *J. Helminth.*, **8** : 19-48.

WALLACE, H. R. (1956) The emergence of larvae from cysts of the beet eelworm *Heterodera schachtii* Schmidt. in aqueous solutions of organic and inorganic substances. *Ann. appl. Biol.*, **44** : 247-282.

WINSLOW, R. D. (1955) The hatching responses of some root-eelworms of the genus *Heterodera*. *Ann. appl. Biol.*, **43** : 19-36.

WINSLOW, R. D. and R. A. LUDWIG, (1957) Studies on hatching stimulation in the beet nematode, *Heterodera schachtii* Schmidt. *Canad. J. Bot.*, **35** : 619-634.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ENVIRONMENTAL CONDITIONS AND FALSE SMUT INCIDENCE IN RICE

K. MANIBHUSHAN RAO*

(Accepted for publication April 10, 1964)

False smut caused by *Ustilaginoidea virens* (Cke.) Tak. is considered to be a minor disease of paddy. Recent reports, however, indicate that the disease causes some amount of loss in the coastal districts of Andhra Pradesh.

At the Central Rice Research Institute, Cuttack, false smut was observed to be very severe in 1962. It was not noted in 1961, though it was present in 1960. Observations were recorded in 1962 in the Genetic Stock collections to observe varietal reaction and in two Agronomic trials to observe whether there was any relationship between the incidence and fertilizer treatments. A comparative study of the meteorological factors of 1960, 1961 and 1962 seasons at the time of flowering of the varieties was also made to correlate the relationship, if any, between the disease incidence and the weather conditions.

Raychaudhuri[3] observed two types of infection of the spikelets. Govinda Rao and Gopala Raju[1] have reported that higher relative humidity and rainfall accompanied by cloudy days during the period of flowering are more favourable for the incidence. Ikegami[2] studied the relation between the manurial levels and the incidence of false smut of rice; he reported that the infection due to the false smut was low on the crop raised under unmanured conditions.

Incidence of false smut on the Genetic Stock : Preliminary observations of the incidence in the Genetic Stock showed that most of the varieties had 1-3 infected smut balls per ear-head and only in a few cases more than 3 grains were infected. Though most of the varieties in this category had 4-8 infected grains only in one or two exceptional cases the infection was higher. In recording observations, therefore, only three classes were recognized *viz.*, without infection, light infection (1-3 smut balls per panicle) and heavy infection (above 4 per panicle).

During the infection studies, it was observed that varieties of medium duration (130-145 days) registered maximum infection, while those of shorter and longer durations were comparatively less affected. Out of the 297 varieties of 130-145 days duration in the Genetic Stock, only 111 varieties were infected, and among these the infection was severe only in 22 varieties. Since the 'severe' incidence was mostly confined to varieties of a particular flowering group, the meteorological factors during the flowering periods of the three successive years were

---

*Present address : University Botany Laboratory, Madras-5.

[1]Govinda Rao, P. and D. Gopal Raju, *Andhra Agric. J.* (1955).

[2]Ikegami, Hatiro, *Res. Buli.* 12, *Faculty Agric. Gija. Univ.*, (1960).

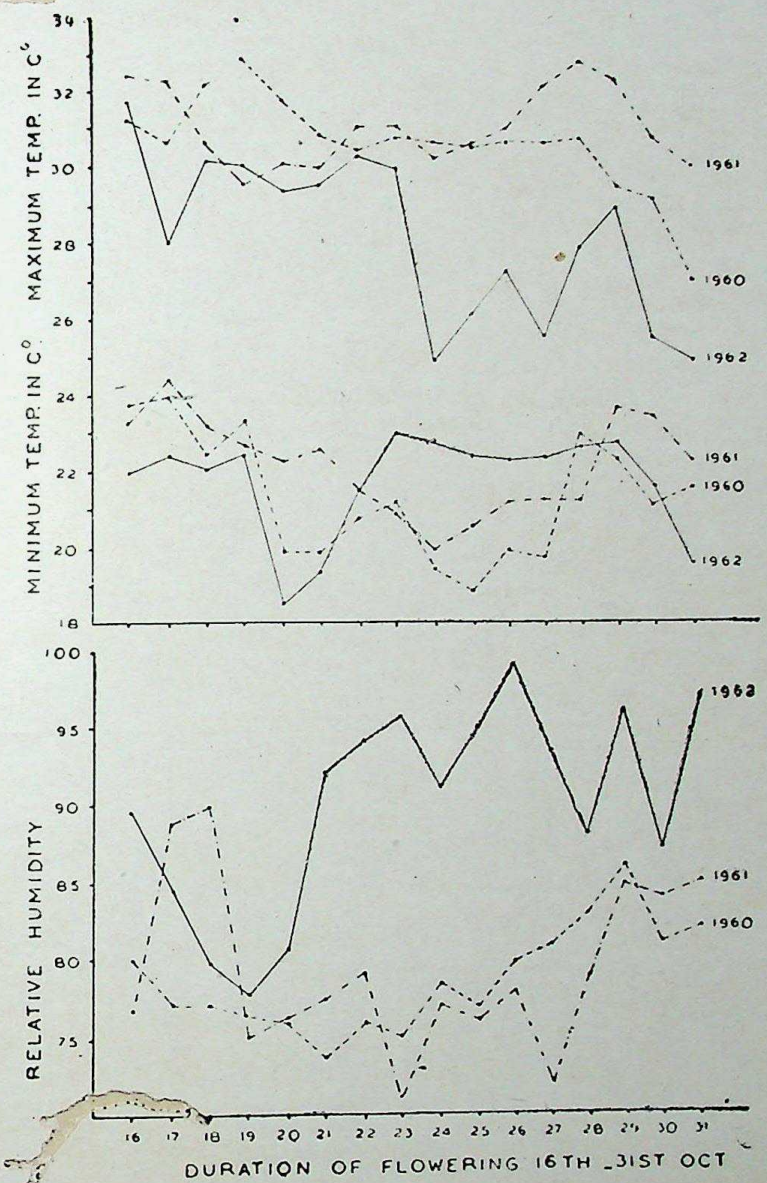
[3]Raychaudhuri, S. P. *J. Indian bot. Soc.* **25**. 145-150 (1946).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

compared to establish the relation between disease incidence and weather conditions.

The flowering date in rice is generally fixed when 50 per cent of the crop is in flower. The process of flower opening in rice panicle continues for a period of 15-20 days. The relevant meteorological factors, like humidity, rainfall, etc. during the fortnight covering the flowering period [illegible] varieties for the three years were compared (Fig. 1).



It showed that flowering period (a fortnight *i.e.*, 16th to 31st October) of the medium duration varieties under study (130-145 days) were characterized by a higher percentage of relative humidity and lower maximum temperature during the year 1962 in comparison to 1961. In 1960 also the percentage relative humidity and the maxima showed a similar trend as in 1962 though the differences were not so marked as between 1961 and 1962. There was also some correlation between the incidence and the number of cloudy days, but not with number of rainy days.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

From the limited data available, it would appear that the actual precipitation has less effect on incidence than cloud amount and low maximum temperature; but lowering of temperature and clouds are phenomena associated with precipitation.

Effect of manurial level on incidence of false smut : Observations were recorded on the incidence of false smut in two manurial experiments conducted at the Institute by the Agronomist. In the first, the effect on yield of different forms of nitrogen was being studied. The objective of the second experiment was to study the effect of time of application of different nitrogenous fertilizers on the yield of rice.

In the first Experiment, T-141 a medium duration variety, maturing within 140 days, was, selected as the test variety. The effect of nitrogen applied as 2 per cent Urea, and Ammonium sulphate, at 30 Kg/N Ha was being compared with the effect on yield obtained from a special solution called 'paddy guard' obtained from Dr. Audich of Punjab. There were 5 different treatments (Table 1) replicated 6 times, with unit of plot size of 4.5×3.5 meters with 16 rows having 18 plants in each row. The crop came to flower in the last week of October and a heavy incidence of false smut was noticed at the time of maturity on 25th October, 1962, just before the harvest of the crop.

Observations were taken by systematic sampling method on two randomly selected plants in every 5th row after cutting the border plants. The ear-heads in each plant, number of infected tillers and the number of infected grains in the infected panicle were noted. The data were analysed statistically and the results are presented in Table 1.

TABLE 1

Treatment	No. of infected plants out of 40 plants	Ratio of infected tillers to total ear-bearing. tillers in 40 plants	No. of grains infected in the infected tillers
2% urea	15 (37.5%)	21/379	59
'Paddy guard' 30 Kg N/Ha	9 (22.5%)	11/349	35
'paddy guard'	4 (10.0%)	7/351	27
30 Kg N/Ha	3 (7.5%)	5/332	15
Control	1 (2.5%)	2/326	5

It may be seen that the highest incidence of false smut was obtained under the treatment : 2 per cent Urea and the lowest in the 'control.'

In the second manurial experiment, the chief variants consisted of 5 varieties and 8 fertilizer treatments with 4 replications as given in Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2

Level of fertilizer applied for main plot	Time of application of fertilizer	No. of infected plants out of 32 plants	Ratio of infected tillers to total ear bearing tillers in 32 plants	No. of grains infected in the infected tillers
1.5 Kg	At the time of transplanting	3 (9.37%)	6/143	19
1.5 Kg	One month after transplanting	7 (21.87%)	9/189	24
1.5 Kg	One month before flowering	9 (28.13%)	14/191	37
0.75 Kg	½ at transplanting, ½ one month after transplanting	2 (6.25%)	5/174	13
0.75 Kg	½ at transplanting, ½ one month before flowering	2 (6.25%)	4/182	12
0.75 Kg	½ one month after transplanting, ½ one month before flowering	4 (12.5 %)	8/163	26
0.50 Kg	⅓ at transplanting, ⅓ one month after transplanting, ⅓ before flowering	7 (21.87%)	11/196	28
Control	Without manure	1 (3.12%)	2/126	4

The object of the experiment was to see the effect of time of application of nitrogen, applied as ammonium sulphate, at different critical periods of crop growth *viz.*, 'transplanting' (7-10 days after transplanting) a month after 'transplanting' and 'flowering' (7-10 days before boot-leaf stage).

The disease incidence was recorded by systematic sampling method, as described in the previous experiment, on one of the five varieties, *i.e.*, AC-2752, which appeared to be most severely infected. The results were analysed statistically as presented in Table 2.

It is evident that the infection was low when no manure had been applied. Among the three 'times of application', there was a gradual increase of infection with later applications, the highest infection being noticed when the fertilizer was applied at the time of flowering.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Application of the fertilizer in the split doses prior to flowering was associated with lower infection than when the application of the entire dose was done at transplanting. However, when the split dose was at the time of transplanting and flowering, the infection was higher than when the entire dose was applied at the time of transplanting.

The infection under the 3 split dose treatments was more or less equal to that in the treatment where the entire dose was applied a month after transplanting. The increase in infection with the various types of application was more or less parallel to the increase in yield obtained with the different treatment - a fact which is in agreement with the popular belief that incidence of the false smut in a crop holds out a promise of a bumper harvest.

From this study, it is clear that environmental conditions especially meteorological factors at the time of flowering and conditions of growth especially nutritional status, have definite influence on the outbreak of false smut. There is apparently also some varietal interaction to false smut, as some varieties appeared to be more susceptible than the others, but on the other hand as many as 186 varieties out of 297 observed *i.e.*, 62.8 per cent remained free from infection under conditions which were apparently favourable for infection.

The study was taken up on the suggestion of Sri S. Y. Padmanabhan, Mycologist, Central Rice Research Institute, Cuttack and the writer is grateful for his guidance. He also wishes to thank Sri I. C. Mahapatra, Agronomist for giving necessary facilities to take observations in his experimental plots; and to Dr. R. H. Richharia, Director, for his keen interest in the study and critical suggestions in the preparation of the paper.

Division of Mycology & Plant Pathology,
Central Rice Research Institute,
Cuttack.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECTS OF VARYING WATER SUPPLY ON THE SUSCEPTIBILITY ON GOMPHRENA GLOBOSA L. PLANTS TO INFECTION WITH POTATO VIRUS X

D. C. SHARMA*

(Accepted for publication April 15, 1964)

The effect of varying soil moisture on the incidence of virus diseases has been studied by Iwanowski (1903), Webb (1927) and Severin (1929) but most observations have been of qualitative nature and have not been able to elucidate the underlying factors. Tinsley (1953) observed that increasing the amount of water supplied to *Nicotiana glutinosa* before inoculation, greatly increased their susceptibility to infection with tobacco mosaic virus (TMV) and tomato bushy stunt virus (TBSV).

Since no information was available for potato virus X, this work was initiated using test plants of *Gomphrena globosa*. These were subjected to differential water supply for different periods before inoculation.

MATERIAL AND METHODS : A ring-spot strain of potato virus X (Sharma and Raychaudhuri, 1962) was used in these experiments. Seedlings of *G. globosa* var. purple were grown in 4″ clay pots containing ¼ Kg. of soil. Three differential water supplies, namely wet, medium and dry were employed as was done by Tinsley (1953). In wet supply, the plants were kept in trays that always contained water so that the soil was continuously wet; in medium and dry supplies, 5 c.c. and 2 c.c., respectively, of water was supplied per plant, per day. Culture of the virus was maintained in *Nicotiana tabacum* var. White Burley, that displayed clear ring-spot symptoms. Freshly expressed sap from recently infected tobacco plants, diluted to 1/10 with water was used as inoculum. Mechanical inoculations were made with an elongated glass spatula without the aid of any abrasive. Four leaves of each of the *G. globosa* plants were inoculated by rubbing their surfaces as uniformly as possible. The inoculated leaves of test plants were washed about half-an-hour after inoculation with a jet of distilled water from a wash bottle. Plants were restored to normal water supply after the treatments were over. Local lesions appeared 7-10 days after inoculation when counts were taken. As the size of leaves varied widely within the treatments, the lesion numbers have been expressed as number per unit (100 sq. cm.), in addition to number per leaf.

To study anatomical changes in leaves of *G. globosa*, leaf pieces of appropriate size were cut from leaves of the same age, from plants that had been subjected to wet or dry supply for 4-week period, and fixed in formalin-acetic-alcohol (70 per cent alcohol) for about 48 hours. Leaf pieces were dehydrated (Johansen, 1940) and embedded in wax,

*Present address : Resources and Scientific Research Division, Planning Commission, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sectioned (t.s.) at 12 μ and stained with Bismark Brown for about 10 minutes, dehydrated in alcohol series and finally mounted in Canada balsam. Camera lucida drawings were made at a magnification × 132.

EXPERIMENTAL : (1) *Effect of continuous wet, medium and dry supply for four weeks* : The data are presented in Table 1.

TABLE 1 : Effect of continuous wet, medium and dry water supply for 4 weeks before inoculation on susceptibility of *G. globosa* plants to infection with potato virus X

	Type of water supply		
	Wet	Medium	Dry
Lesions per leaf	9	6	1
Lesions per 100 sq. cm.	102	15	8

Results of two experiments show that susceptibility of plants to viral infection increases with comparative increase in amount of water supplied before inoculation (Fig. 1).

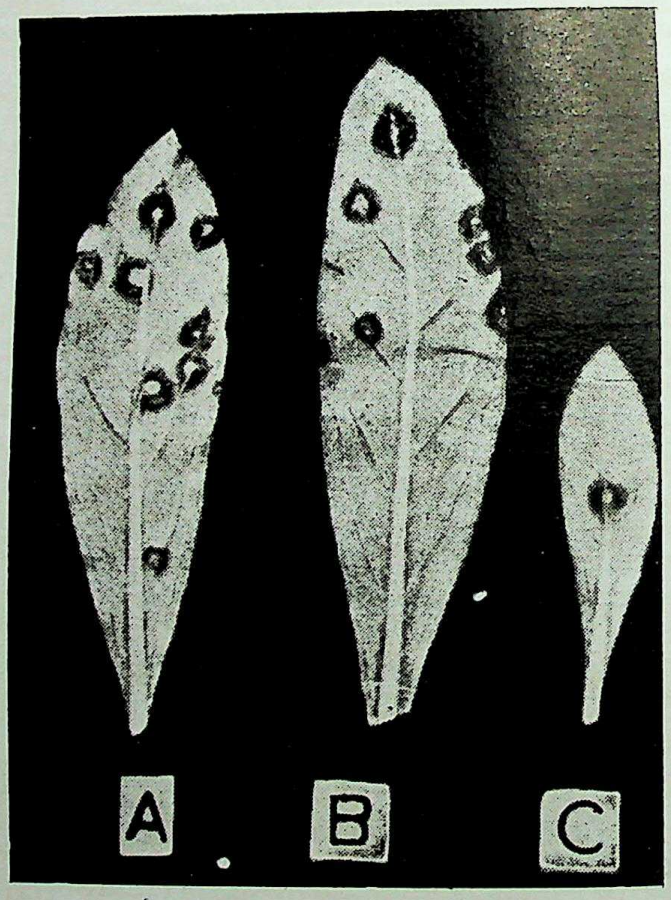


Fig. 1. Number of local lesions per leaf affected by differential water supply. A - Wet supply, B - Medium supply and C - Dry supply for 4 weeks.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(2) *Effect of alternating periods of wet and dry supply* : Two experiments were undertaken in which plants subjected to wet and dry supply for 4 weeks prior to inoculation were compared with plants that were first subjected to wet or dry supply for either the first or the last 2 weeks as the case may be. A clear difference in the susceptibility of the plants was evident when the water supply was changed from wet to dry or *vice versa*. Type of final water supply administered immediately before inoculation influenced the host susceptibility, (Fig. 2), as also the leaf size. The data are presented in Table 2.

TABLE 2 : Effect of alternating periods of wet and dry supplies on the susceptibility of *G. globosa* plants to infection with potato virus X

	Type of water supply			
	Wet 4 weeks	Wet 2 weeks Dry 2 weeks	Dry 4 weeks	Dry 2 weeks Wet 2 weeks
Lesions per leaf	8	2	1	6
Lesions per 100 sq. cm.	112	86	11	106

It will be noticed that varying amount of water supply considerably influenced the degree of susceptibility of the plants. The different treatments affected the height of the plants and size of the leaves as well. Plants receiving wet supply for 4 weeks had large and thin leaves with light green colour. While those subjected to dry supply for the same period had comparatively thicker and smaller leaves with dark-bronze colour.

A change from wet to dry supply or *vice versa*, after an initial 2 week supply of either of these, brought about changes in host susceptibility and leaf size.

(3) *Water supply and leaf structure* : Differences noticed in external appearance of leaves of *G. globosa* subjected to different amounts of water supply gave an indication that their internal structure was likely to differ. Figs. 3 and 4 show the anatomy of comparable leaves of plants subjected to wet and dry supplies. Marked differences are seen in these two figures. The leaves borne by plants subjected to wet supply for 4 weeks developed thin cuticle, larger epidermal cells as also chlorenchyma and spongy parenchyma which were more loosely arranged and leaf as a whole, was thicker. The leaves borne by plants subjected to dry supply developed comparatively thicker cuticle and smaller cells arranged in a compact manner in the tissues.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

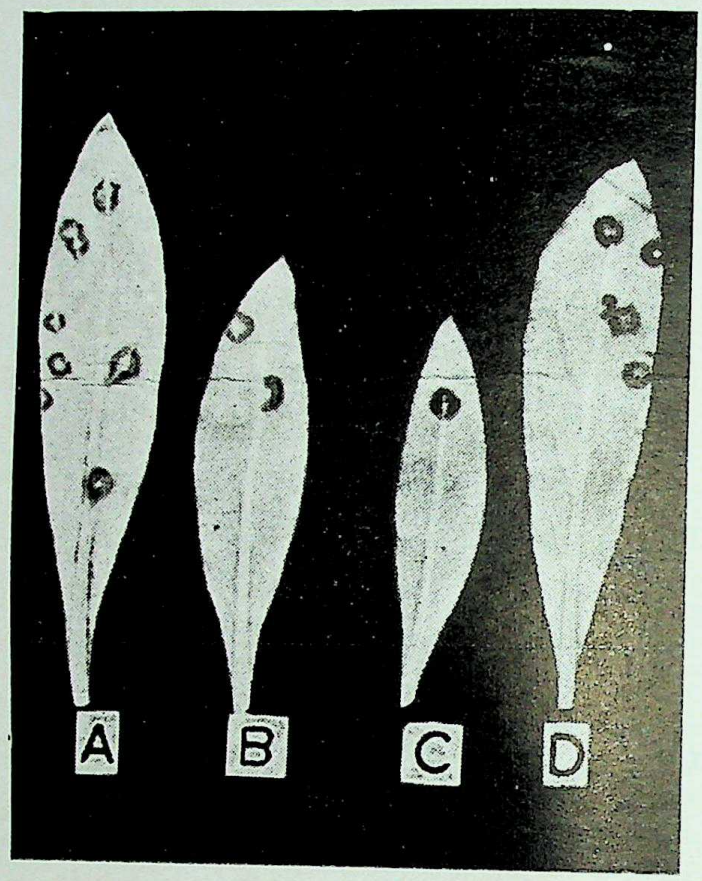


Fig. 2. Number of local lesions per leaf as affected by alternating wet and dry supplies. A - Wet supply for 4 weeks, B - Wet supply for 2 weeks followed by dry supply for 2 weeks, C - Dry supply for 4 weeks, and D - Dry supply for 2 weeks followed by wet supply for 2 weeks.

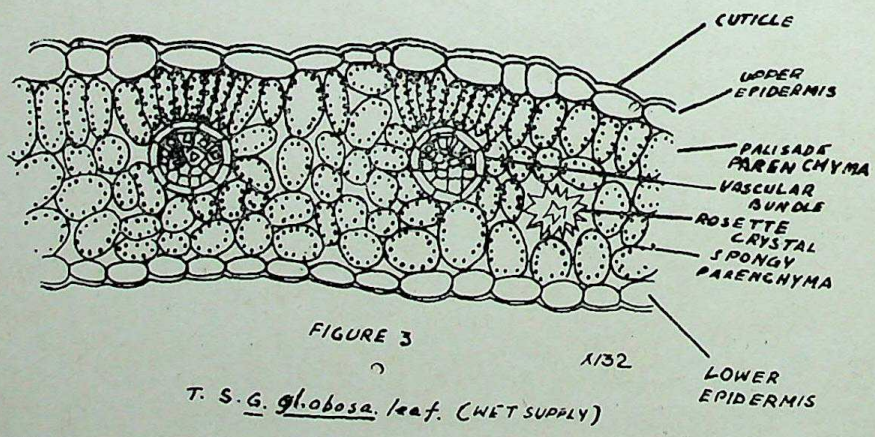


Fig. 3 T. S. of leaf of *Gomphrena globosa* (Wet supply for 4 weeks)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

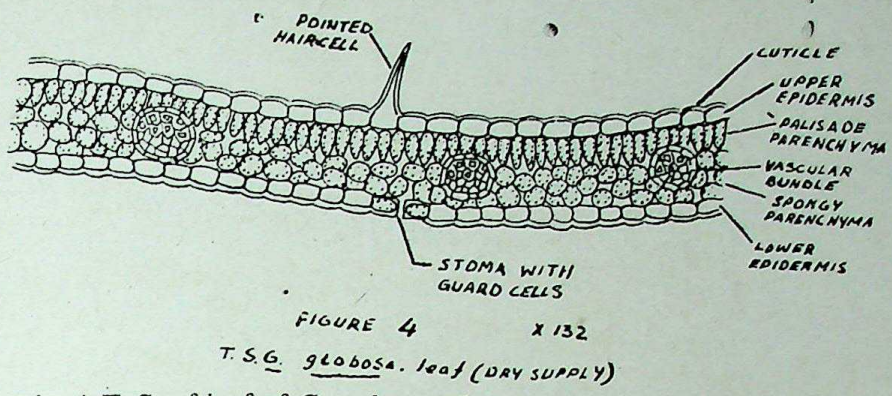


Fig. 4 T. S. of leaf of *Gomphrena globosa* (Dry supply for 4 weeks)

DISCUSSION : In the experiments described in this paper, potato virus X produced results very similar to those obtained by Tinsley (1953) with TMV and TBSV on *Nicotiana glutinosa*. The susceptibility of *Gomphrena globosa* depended on varying amounts of water (wet, medium and dry supply) administered before inoculation. The lesions produced on *G. globosa* were pronounced, well defined, closely aggregated and severely necrotic in plants given wet supply. It is clearly demonstrated that wet supply of test plants facilitates the establishment and multiplication of introduced viral particles. Detailed studies on the relationship between host cell physiology and viral invasiveness and multiplication which favour the latter to benefit in plants supplied with excess of water, will result in a better understanding of the host-parasite relationship in viral infection.

SUMMARY

The susceptibility of *Gomphrena globosa* plants to infection with potato virus X were influenced appreciably by subjecting them to differential water supply (wet, medium and dry) for a period of 4 weeks before inoculation. A marked difference was seen in susceptibility as also in leaf size, texture, and colour of the test plants. An alternation of water supply from wet to dry or *vice versa* in either the first or second 2-week period, of the entire 4-week treatment, also brought about changes in susceptibility to infection. Wet and dry supplies induced marked changes in leaf structure and cell-size. Test plants showed pronounced, well-defined and severe necrotic lesions due to infection with virus in plants given wet supply.

ACKNOWLEDGEMENTS : The author is indebted to Dr. R.S. Vasudeva, former Head and to Dr. B.L. Chona, present Head, for kindly providing facilities and for their interest in the work. The author is also grateful to Dr. S.P. Raychaudhuri, Professor of Mycology and Plant Pathology for suggesting the problem and for his guidance during the course of investigation.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

IWANOWSKI, D.J.V.V. (1903) Ueber die Mosaik Karankheit der tabakspflanze *Z. Pflkrankh.*, **13** : 1-14.

JOHANSEN, D.A. (1940) *Plant Microtechnique.* McGraw Hill and Co., London, New York.

SEVERIN, H.H.P. (1929) Curly top symptoms on the sugarbeet. *California Agri. Expt. Sta. Bul.* **465.**

SHARMA, D.C. and S.P. Raychaudhuri (1962) Occurrence of a ring spot strain of Potato virus X. *Indian Phytopath.*, **15** : 250-253.

TINSLEY, T.W. (1953) The effect of varying the water supply of plants on their susceptibility to infection with viruses. *Ann. Appl. Biol.*, **40** : 750-760.

WEBB, R.W. (1927) Soil factors influencing the development of the mosaic disease of winter wheat, *J. agri. Res.*, **35** : 587-614.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECT OF SOME VITAMINS ON THE GROWTH OF FUSARIUM OXYSPORUM F. CUMINI ISOLATES

B. L. MATHUR, L. C. SHARMA AND N. PRASAD[1]

(Accepted for publication April 16, 1964)

Essentiality of vitamins has been observed for various fungi which normally do not grow on synthetic media. Vitamin deficiencies of many species of fungi have been reviewed by Hawker (1950) and Lilly and Barnett (1951). However, comparatively little is known about the vitamin deficiencies of strains within the same species. Lilly and Barnett (1951) noted differences in vitamin requirements between 2 isolates of *Botryotinia convoluta*, 4 isolates of *Ciboria acerina* and 2 isolates of *Stromatinia smilacinae*. Mathur, Lilly and Barnett (1949) observed them among 4 strains of *Colletotrichum lindemuthianum*, and Barnett (1956) in different strains of *Endoconidiophora fagacearum*. Most of the species of the genus *Fusarium* studied showed no deficiency for any vitamin (Lilly and Barnett, 1951; Robbins and Kavanagh, 1942). Hawever, Robbins and Ma (1941) and Prasad (1949) observed increased growth in one strain each of *Fusarium avenaceum* and *F. solani f. cucurbitae* respectively with biotin. Elliott (1949) reported that the growth of one vitamin self-sufficient isolate of *Fusarium avenaceum* was reduuced by addition of thiamine to the vitamin-free media. Prasad and Patel (1958) observed that all the isolates of cumin wilt *Fusaria* from Gujarat were self-sufficient in vitamins. Experiments were conducted to know whether the general observation for *Fusaria* species holds good for the isolates of cumin wilt *Fusaria* of Rajasthan. (Mathur. 1960)

MATERIAL AND METHODS : Experiments were carried out with various isolates of *Fusarium oxysporum f. cumini* (Mathur, 1960). The basal medium, Richards solution consisted of KNO_3 : 10 gms; KH_2PO_4 : 4.0 gms; $MgSO_4 . 7H_2O$: 2.5 gms; Sucrose : 50 gms; $FeCl_3$: traces; double-distilled water in Pyrex still to make 1,000 ml. Glassware used in the experiments was cleaned with chromic-sulphuric acid mixture and was washed with tap water and then distilled water. Double-glass distilled water was used to prepare media and vitamin solutions. The impurities from the media were removed through the calcium carbonate method suggested by Steinberg (1935) and then $FeCl_3$ was added. The pH of the medium was adjusted to 6.0 before autoclaving with N/20 NaOH or acetic acid. Vitamins were added to the basal medium at the following rates per litre :—Thiamine 100/ug.; biotin-6/ug.; pyridoxine-50/ug.; riboflavin-100/ug.; folic acid-100/ug.; and inositol-2.5 gms. The cultures were grown either in 90 mm. petri-plates on media solidfied with Difco agar or on solutions in Pyrex Erlenmeyer flasks (100cc), the flasks were plugged with cotton wrapped in cigarette paper. Inoculation was carried out with germinating single spores from Difco agar of highest purity and incubated at room temperature. The mycelial mats from liquid cultures were harvested and weighed after 10 days. Growth in petri-plates

[1]At present Associate Dean, S.K.N. Agriculture College, Jobner (Jaipur).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

was visually rated and colony diameters taken in two directions after one week. In liquid media, the fungal mats were filtered on Whatman filter paper No. 42, washed thrice with distilled water, dried at 60°C. for 24 hours, cooled and weighed. The mean values of 3 to 4 replications were recorded.

RESULTS : *Vitamin deficiencies in different strains* : Data presented in Table 1 reveald that isolate 1 is partially deficient for biotin, riboflavin and folic acid. Inositol appeared to be without any stimulatory effect, while thiamine and pyridoxine reduced the growth. Combination of all the vitamins induced growth to nearly double the control. Growth of isolate 4 and 41 was substantially increased by addition of biotin, folic acid, riboflavin, and inositol; thiamine and pyridoxine being without any effect. The combination of all the vitamins increased the growth in isolate 4, whereas in isolate 41, it was increased by two and a half times. Isolate 27 is deficient for all the vitamins tested; there was increase in growth with all the individual vitamins including thiamine and pyridoxine. The latter 2 vitamins have not been found to increase growth in other isolates tested, the mixture induced about four times as much growth as in control. Growth in isolate 33 and 40 was much reduced by addition of thiamine and pyridoxine; addition of folic acid, inositol and combination of all vitamins increased the growth. In almost all isolates growth increased considerably by addition of folic acid. Increase in mycelial yield in presence of thiamine in isolates 27 and 41 is an exception.

TABLE 1 : Weight of mycelial mats of *Fusarium oxysporum f. cumini* on Richards medium supplemented with different vitamins

Vitamins	Growth expressed in mg. of dry weight of mycelial mats in 20 ml. solution after 20 days of inoculation					
	1	4	27	33	40	41
No vitamin	62.2	118.2	43.3	41.7	42.2	63.2
Thiamine	49.1	117.7	63.1	12.2	36.5	64.1
Biotin	76.1	155.6	67.3	45.1	56.6	81.8
Pyridoxine	44.1	113.8	67.4	32.7	3.4	61.9
Riboflavin	87.1	154.9	74.0	39.8	24.2	93.2
Folio acid	112.3	183.9	83.7	54.9	94.1	110.8
Inositol	62.5	129.9	64.2	62.1	84.8	96.6
All Vitamins	119.0	154.6	157.4	65.4	127.1	158.1

Effect of thiamine and biotin added singly on the growth of different Isolates : In solidified media, it is clear from fig. 1 that addition of thiamine reduced the growth in isolates 33 and 40, whereas biotin

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

increased the growth in case of isolate 33 only. No increase in growth is visible on solidified media containing biotin and thiamine both except that of isolate 33.

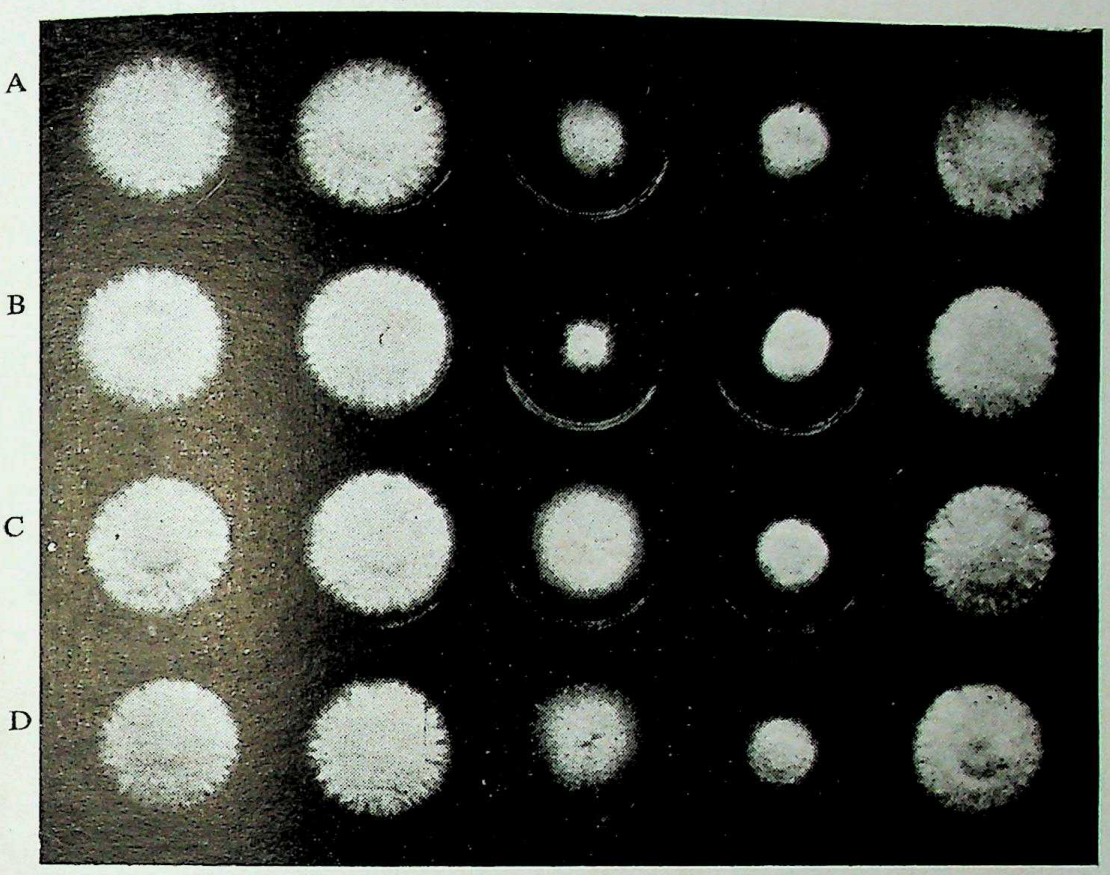


Fig. 1. Growth of various isolates on vitamins. From top to bottom :—
A No vitamin. B Thiamine. C Biotin. D Thiamine plus biotin

In liquid media containing thiamine, the growth was reduced in isolate 33 and 40 considerably, but in other cases slightly, whereas, in isolate 41 growth slightly increased. Biotin increased the the growth in almost all cases. Mycelial yield of isolate 40 in presence of thiamine plus biotin was reduced as compared to control. In some cases considerable increase in mycelial weight in media containing thiamine and biotin both was observed, (Table 2). Prasad and Patel (1958) made a

TABLE 2 : *Mycelial weight of different isolates with thiamine and biotin*

Vitamins	Mycelial—Weight in mg/20 ml. solution after 10 days				
	1	4	33	40	41
No vitamin	96.5	140.3	55.7	68.3	104.8
Thiamine	84.2	136.1	27.5	54.7	109.5
Biotin	102.1	169.5	72.1	76.3	121.6
Thiamine+Biotin	111.8	163.2	69.3	67.3	129.2

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

study of cumin wilt *Fusaria* for their vitamin requirement on solid media only, which might be the reason for their concluding that cumin wilt *Fusaria* are autotrophic for vitamins. *Fusarium* sp. can utilize agar or the impurities contained in it.

DISCUSSION : Robbins and Kavanagh (1942) recognized two kinds of heterotrophy *viz.*, complete and partial. Both may be for one vitamin or multiple for more than one vitamins. The widely worked out fungus *Phycomyces blakesleeanus* is a classical example of complete deficiency for a single vitamin, thiamine (Schopfer, 1943). In the present case the deficiency is partial, since the growth of some of the isolates increases in presence of biotin, riboflavin, folic acid and inositol; in absence of these vitamins the various isolates of this fungus still attained good growth.

Besides these, the reducing effect on the growth of certain fungi by vitamins has also been reported. The depressing effect of thiamine on three strains of *Colletotrichum lindemuthianum*, five strains of *Endoconidiophora fagacearum* one strain of *Fusarium avenaceum* has been reported by Mathur, Lilly and Barnett (1949), Barnett (1956) and Elliot (1949) respectively. Depressing effect due to thiamine, pyridoxine and inositol in certain strains of *Endoconidiophora fagacearum* has been reported by Hoffman (1954). Similarly depressing effect due to thiamine, pyridoxine, and riboflavin in certain strains of *Fusarium oxysporum f. cumini* have been observed. Schopfer and Guillound (1945) found that the depressing of growth of *Rhizopus suinus* caused by thiamine was overcome by inositol. In cumin wilt *Fusaria* depressing of growth caused by thiamine was overcome by biotin in most cases.

SUMMARY

Studies on the vitamin requirements of different isolates of *Fusarium oxysporum f. cumini* revealed that the fungus is partially heterotrophic. Although good growth was obtained even in absence of vitamin, it increased considerably with addition of biotin, riboflavin, folic acid, and in some case with inositol. Addition of thiamine and pyridoxine was either without effect or depressed the growth. The depressing effect of thiamine was generally counter-balanced by the addition of biotin.

ACKNOWLEDGEMENT : The authors wish to express their thanks to Shri R. L. Mathur, Plant Pathologist, Rajasthan, Udaipur for help in many ways and the Director of Agriculture, Rajasthan, Jaipur for providing laboratory facilities.

Plant Pathological Section,
Rajasthan, Jaipur.

REFERENCES

BARNETT H. L. (1956) Variation in oak wilt fungus. *Endoconidiophora fagacearum. Proc. W. V. Acad. Sci.*, **27**, 25-29.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ELLIOTT, F. S. (1949) Effect of vitamins on the growth of some graminicolous species of *Helminthosporium* and *Fusarium*. *Proc. W. V. Acad. Sci.*, **20** : 65-68.

HAWKER, L. E. (1950) *Physiology of fungi*. Univ. Press, London.

HOFFMAN, P. F. (1954) Physiology of *Endoconidiophora fagacearum* Bretz. I Factors influencing growth and toxin production. *Iowa State College J. Sci.*, **29** : 27-38.

LILLY, V. G. AND H. L. BARNETT, (1951) *Physiology of fungi*. McGraw Hill Co., New York.

MATHUR, B. L. (1960) *Studies on* Fusarium *wilt of cumin*. Ph. D. thesis, Rajasthan University.

MATHUR, R. S. V. G. LILLY, AND H. L. BARNETT, (1949) Partial vitamin deficiencies in four strains of *Colletotrichum lindemuthianum*. *Indian phytopath.*, **2** : 160-165.

PRASAD, N. (1949) Variability of the cucurbit rot not fungus *Fusarium solani f. cucurbitae Phytopathology*, **39** : 132-142.

PRASAD, N. AND P. N. PATEL, (1958) Metabolism of *Fusarium oxysporum f. cumini*. *Symposium on recent advances in the study of plant metabolism*, University of Allahabad.

ROBBINS, W. J, AND F. KAVANAGH, (1942) Vitamin deficiencies of the filamentous fungi. *Bot. Rev.*, **8** : 411-471.

ROBBINS, W. J. AND R. MA, (1941) Biotin and the growth of *Fusarium avenaceum*. *Bull. Torrey bot. Cl.*, **68** : 446-462.

SCHOPFER, W. H. (1943) *Plants and Vitamins*. Chronica Botanica Co. Waltham, Mass., U.S.A.

*SCHOPFER, W. H. AND M. GUILLOUND, (1945) Lac culture d' Eremothecium Ashbyii en milien synthetique. *Experientia*, **1** : 1-3.

STEINBERG, R. A. (1935) The nutritional requirements of the fungus *Aspergillus niger*. *Bull. Torrey bot. Cl.*, **62** : 81-90.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON HOST RANGE AND HISTOPATHOLOGY OF ROOT-KNOT INFECTIONS CAUSED BY MELOIDOGYNE JAVANICA

M. BALASUBRAMANIAN AND G. RANGASWAMI

(Accepted for publication April 20, 1964)

The root-knot nematodes which cause reductions of various plant parts, particularly of the roots are well known. Studies were undertaken to examine the host range, gall characters and histological changes in the hosts due to *Meloidogyne javanica* (Treub) Chitwood and the results are presented here.

MATERIAL AND METHODS : For host range studies, 30 plant species belonging to 14 different families were inoculated, following the procedure described in the earlier report (Rangaswami *et al.*, 1960). Under each species 16 plants were included, 8 for the control and 8 for the inoculation. The roots were examined periodically for the presence of nematodes. The development and egg production as well as the host reactions to nematode infection were recorded. The relative root-knot index for some important hosts was worked out, following the methods of Smith and Taylor (1947). To find out the most susceptible period of plant growth for infection by *M. javanica*, seeds of a local variety of bhendi (*Abelmoschus esculentus* Moench) were surface sterilized with 0.1 per cent mercuric chloride and sown in steam sterilized soil in 6 inch pots. Equal quantities of nematode suspension were added to the soil at periodical intervals, *i.e.*, (i) mixed with the soil at the time of sowing, (ii) added to the soil 15 days after seed germination and (iii) added to soil 30 days after seed germination. The plants were pulled out from both the inoculated and control plants, root samples selected at random, fixed in Randolph's modification of Navashin fixative (Dropkin and Nelson, 1960) and maintained in the medium until they were taken up for sectioning. Thin transverse sections of the roots were cut and stained with Safranin-Fast green schedule and permanent mounts prepared following the procedure given by Johansen (1940). The damage caused by the nematodes to the various parts of roots was differentiated by staining in Eosin-lactophenol. The giant cells were classified, following the method of Dropkin and Nelson (1960).

EXPERIMENTAL RESULTS : (i) *Host range* : Out of the 30 plant species tested, 19 exhibited the root-knots; various stages of *M. javanica*, including females with egg sacs, were noted in the tissues (Table 1). Plants belonging to Amaranthaceae, Capparidaceae, Euphorbiaceae, Labiatae, Leguminosae, Malvaceae, Nyctaginaceae, Solanaceae, and Tiliaceae took infection but not those belonging to families Aizoaceae, Aristolochiaceae, Compositae, Convolvulaceae and Verbenaceae. However, *Croton sparsiflorus* Morong and *Phyllanthus niruri* L. of Euphorbiaceae, *Malvastrum coromandelianum* Garcke and *Pavonia zeylanica* Cav. of Malvaceae and *Tridax procumbens* L. of *Compositae* did not take infection.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Host range of *M. javanica* as observed under artificial inoculation (Results obtained 45 days after inoculation)

Name of the host plant	Family	Infected or not
Abelmoschus esculentus Moench	Malvaceae	+
Acalypha indica L.	Euphorbiaceae	+
Amaranthus viridis L.	Amaranthaceae	+
Aristolochia bracteata Retz.	Aristolochiaceae	—
Boerhaavia diffusa L.	Nyctaginaceae	+
Cassia occidentalis L.	Leguminosae	—
Cleome viscosa L.	Capparidaceae	+
Convolvulus arvensis L.	Convolvulaceae	+
Corchorus acutangulus Lan.	Tiliaceae	+
Croton sparsiflorus Morong.	Euphorbiaceae	—
Eclipta alba Hassk.	Euphorbiaceae	+
Euphorbia hirta L.	Compositae	—
Gomphrena decumbens Jacq.	Amaranthaceae	+
Gynandropsis pentaphylla DC.	Capparidaceae	+
Lippia nodiflora Mich.	Verbenaceae	—
Lycopersicon esculentum Mill	Solanaceae	+
Malvastrum coromandelianum Garcke	Malvaceae	—
Nicotiana tabacum L.	Solanaceae	+
Ocimum sanctum L.	Labiatae	+
Pavonia zeylanica Cav.	Malvaceae	—
Phaseolus trilobus Ait.	Leguminosae	+
Phyllanthus niruri L.	Euphorbiaceae	—
Sesbania aculeata Poir.	Leguminosae	+
S. speciosa Taub.	Solanaceae	+
Solanum melongena L.	Solanaceae	+
S. nigrum L.	Solanaceae	+
S. trilobatum L.	Solanaceae	+
Trianthema portulacastrum L.	Aizoaceae	—
Tridax procumbens L.	Compositae	—
Vernonia cinerea Less	Compositae	—

+infected.
—not infected.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(ii) *Reduction in tap-root length* : The reduction in tap-root length varied from 12.61 to 47.65 per cent in the various hosts (Table 2). The maximum reduction was 47.65 per cent in the roots of *Ocimum sanctum*. and the lowest was in *Sesbania aculeata* with 12.61 per cent. Even in the highly susceptible hosts like *A. esculentus* and *Nicotiana tabacum* the reduction was only about 22 per cent. The root length reduction varied widely even in the plants belonging to the same family, *viz.*, 28.65 per cent in *Acalypha indica* and 46.10 per cent in *Eclipta alba*. The plants belonging to the same genus, *Sesbania*, differed in their reaction to the nematode infection: in *S. aculeata* the reduction was 12.61 per cent and in *S. speciosa* it was 19.13 per cent.

TABLE 2 : Percentage of root length reduction in some hosts due to infection by *M. javanica*

Name of the host plant	Average length of tap-root in cm.		Per cent reduction
	Healthy	Infected	
A. esculentus	14.4	11.1	22.91
A. indica	17.1	12.1	28.65
B. diffusa	39.3	27.3	31.60
C. viscosa	11.1	7.4	33.33
C. acutangulus	35.8	22.4	37.80
E. alba	37.8	20.4	46.10
G. decumbens	14.1	10.7	24.11
G. pentaphylla	17.2	14.3	16.82
L. esculentum	21.7	15.76	27.84
N. tabacum	19.80	15.21	22.21
O. sanctum	43.75	22.90	47.65
P. trilobus	11.10	8.70	21.62
S. aculeata	11.10	9.70	12.61
S. speciosa	16.20	13.10	19.13
S. melongena	16.10	10.70	33.54
S. nigrum	11.70	10-21	12.73
S. trilobatum	18.10	15.10	16.57

(iii) *Relative root-knot index* : The relative root-knot index of *M. javanica* on bhendi, brinjal, tomato, tobacco and sugarcane is given in Table 3. The results indicate that all these five hosts are more or less equally susceptible to *M. javanica*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Relative root-knot index in some hosts of *M. javanica* (Average of ten plants under each host)

Host plant	Total No. of roots/ plant	Total No. of roots that bear galls	Per cent of roots that bear galls	Root-knot index*
A. esculentus	22	13	58.5	2.3
L. esculentum	26	22	85.0	3.4
N. tabacum	31	24	77.5	3.1
S. officinarum	26	19	72.5	2.9
S. melongena	42	33	80.0	3.2

*Percentage of

*roots infected		Index No.
1—25 per cent	...	1
26—50 per cent	...	2
51—75 per cent	...	3
76—100 per cent	...	4

(*iv*) *Age of the plant and intensity of nematode infection* : When the plants were inoculated 15 days after germination, the maximum gall formation was found and the relative root-knot index reached the maximum of 3.6 on the 60th day, followed by 2.5 due to inoculation made on the 30th day and 1.9 due to inoculation made on the 45th day. The maximum number of galls developed in the plants, which were inoculated on the 15th day after sowing, whereas the minimum number of galls was noticed when they were inoculated on the 45th day.

(*v*) *The general anatomical deviations due to* M. javanica : In all the hosts the epidermis and cortex seem to be damaged, except in *S. aculeata* where the damage to the epidermis was not significant. In all the eight hosts the pericycle region showed damage due to the nematode attack, but in the case of *S. officinarum* damage to the cells was only minor. The vascular bundles were not much damaged as compared to the damage to cortex and pericycle where large cavities were formed. The cavities were small-sized in *S. officinarum* and relatively bigger in *S. melongena*. These cavities were seen either in the cortex and/or in the pericycle.

(*vi*) *The nature of giant cells caused by* M. javanica : In the present studies all the eight host species exhibited giant cells in the cortex and sometimes in the pericycle and stele regions. In *S. officinarum* giant cells were always connected to the endodermis and they were formed in the cortex in such a manner as to push the endodermis to one side. In all the eight hosts the giant cells were found to fall invariably under the fourth type of Dropkin and Nelson (1960). In *S. officinarum* the giant cells were smaller than those of other hosts.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The number of cells which go to form the multinucleate giant cells by coalescence also varied from plant to plant. In the roots of *A. esculentus* a maximum of only seems four cells to coalesce to form the giant cell. In *S. officinarum* seven such cells were required, in *C. acutangulus* and *S. melongena* four to five cells and in *N. tabacum* four cells were found to form the giant cell. The thickness of cell wall varied with the host species, indicating thereby that the different plants reacted differently to form the giant cells.

(*vii*) *Frequency of giant cell formation in different hosts by* M. javanica : The total number of giant cells per section in host plant was counted so as to estimate the number of loci where the nematodes inserted their stylets to get their nourishment. For that purpose, in each case 10 sections, selected at random, were examined. The total numbers of giant cells recorded in the hosts are : *A. esculentus*—7, *L. esculentum*—20, *N. tabacum*—14, *S. officinarum*—11 and *S. melongena*—13.

DISCUSSION : More than a thousand host plants are reported for the genus *Meloidogyne*, and about two hundred for the species, *M. javanica* (Jones, 1959). Of the nineteen hosts recorded for *M. javanica* in the present studies, eleven are new ones. *G. pentaphylla* and *N. tabacum*, known in other countries as hosts for *M. javanica*, are being recorded in India for the first time. Though *M. javanica* is capable of infecting large number of plant species, it has host preferences within the plant families and within the genera, as indicated in the present studies, which is in close agreement with the earlier reports (Sasser, 1954; Seinhorst, 1961). However, *P. niruri* and *C. sparsiflorus* reported as hosts for *M. javanica* by Singh (1960) and Nirula and Kumar (1963) respectively, failed to get infected in these studies. This may probably be due to the existence of physiologic races or some other factors.

As observed in the present studies, the reduction of tap-root length need not always be attributed to the intensity of nematode infection. There is evidence to show that soft textured roots contained more nematodes than those with hard textured ones; the roots of *O. sanctum* are comparatively soft textured than those of *A. esculentus*, and there were more root-knots in the former than the latter. The total number of galls and their size and shape varied with the host plants, indicating thereby that the plant-nematode relationships differed considerably. In *A. indica* and *A. esculentus* most of the galls were found on the tap-root, whereas in *E. alba* mostly the secondary roots were galled. Godfrey and Oliveria (1932) reported that in pineapple the primary roots escaped serious injury and Reynolds (1955) found that finer feeder roots were attacked by *M. javanica*. Dropkin (1959) reported that in soybean, *M. arenaria* (Neal) Chitwood galls were confined to the tap root and he indicated a differential attractiveness of the tap root to the larvae of *M. arenaria*.

In the histopathological studies it was brought out that the giant cells were found in the cortex of all the plant hosts. This is in agreement with the previous reports (Christie 1936; Dropkin and Nelson 1959; Bird 1961). In addition, the giant cells were seen in pericycle in *A. esculentus*, *C. acutangulus*, *G. decumbens*, *N. tabacum* and *S. melongena*. In *S. officinarum*, where the giant cells are always connected with

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the endodermis, there are indications for the differential behaviour of the roots to form the giant cell. The type of giant cells in all the roots examined, except in sugarcane, fall under the fourth type described by Dropkin and Nelson (1960), in being large and smooth, with uniformly stained contents and thick cell walls.

SUMMARY

The root-knot nematode, *Meloidogyne javanica* isolated from sugarcane was found to infect, on artificial inoculation, nineteen out of thirty hosts tested. Of these, *Amaranthus virids*, *Boerhaavia diffusa*, *Convolvulus arvensis*, *Corchorus acutangulus*, *Eclipta alba*, *Gomphrena decumbens*, *Ocimum sanctum*, *Phaseolus trilobus*, *Sesbania aculeata* and *S. speciosa* are new hosts for the nematode and *Gynandropsis pentaphylla* and *Nicotiana tabacum* new records for India.

Morphological changes in the root system caused by *M. javanica* varied in different hosts; the tap-root length reduction varied from 12.61 to 47 per cent; the total number of galls per plant ranged from 10 to 500 or more; the gall size, shape and position on the root system varied with the host plant. The total number of galls did not give a correct indication of the severity of infection, whereas the root-knot index, based on the percentage of roots infected together with the reduction in root length, gave a more realistic idea of relative susceptibility of host plants to nematode infection. In *A. esculentum* the maximum infection was observed when *M. javanica* was inoculated on the 15th day after seed germination, whereas inoculations on the 30th and 45th day caused relatively lesser infections.

Studies on the histological changes caused by the nematode in eight of the susceptible hosts showed that the epidermis, cortex and pericycle were damaged, and characteristic cavities and giant cells formed in various tissues of the host root.

Microbiology Laboratory,
Department of Agriculture,
Annamalai University,
Annamalainagar,
Madras State.

REFERENCES

BIRD, A. F. (1961) The ultra-structure or histo-chemistry of nematode induced giant cells. *J. Biophys. and Biochem. Cytol.*, **11** : 701-715.

CHRISTIE, J.R. (1936) The development of root-knot nematode gall. *Phytopathology*, **26** : 1-22.

DROPKIN, V. H. (1959) Varietal response of soybeans to *Meloidogyne*; a bioassay system for separating races of root-knot nematodes. *Phytopathology*, **49** : 18-23.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

DROPKIN, V. H. AND P. E. NELSON, (1959) The pathogenicity of *Meloidogyne* spp. (root-knot nematode). *Proc. 9th Inter. Cong. Botan.*, **2** : 97.

DROPKIN, V. H. AND P. E. NELSON, (1960) The histopathology of root-knot nematode infections in soybeans. *Phytopathology*, **50** : 442-447.

GODFREY G. H. AND J. OLIVERIA, (1932) The development of root-knot nematode in relation to root-tissue of pineapple and cowpea. *Phytopathology*, **22** : 325-348.

JOHANSEN, D. A. (1940) *Plant Microtechnique*. McGraw Hill Book Co., Inc., New York, U.S.A.

JONES, F. G. W. (1959) *Plant Pathology Problems and Progress*. pp. 395-411. 1908-1958. The Univ. of Wiswnsin Press, Madison.

NIRULA, K. K. AND R. KUMAR, (1963) Collateral host plants of root-knot nematodes. *Curr. sci.*, **32** : 221-222.

RANGASWAMI, G., V. N. VASANTHARAJAN, AND R. VENKATESEN, (1960) The occurrence of root-knot nematodes on sugarcane and on some weeds. *Curr. sci.*, **29** : 236-237.

REYNOLDS, H. W. (1955) Varietal susceptibility of alfalfa to two species of root-knot nematode. *Phytopathology*, **45** : 70-72.

SASSER, J. N. (1954) Identification and host parasitic relationship of certain root-knot nematodes (*Meloidogyne* spp.), *Bull. Md. Agric. Expt. Sta., A. 77 (Tech.)* pp. 31.

SEINHORST, J. W. (1961) Plant nematode interrelationships. *Annu. Rev. Microbiol.*, **15** : 177-186.

SINGH, K. (1960) Suspected plant parasitic nematodes in certain sugarcane soils of Madras State. *Indian Phytopath.*, **13** : 181-182.

SMITH, A. L. AND L. A. TAYLOR, (1947) Field methods of testing for root-knot infestation. *Phytopathology*, **37** : 85-93.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SOME OBSERVATIONS ON LINSEED WILT

L. B. GOEL AND GOPAL SWARUP

(Accepted for publication July 28, 1964)

Wilt is one of the most serious diseases of linseed, but so far it has not received much attention. It was first reported by Pearl in 1923 from the erstwhile Central Provinces and Berar. Later, the occurrence of the disease has been reported by several other workers, which indicate that the wilt disease, caused by *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hansen is responsible for much heavier losses than hitherto considered. In view of the growing importance of the oilseed crops in the national economy, as well as the lacuna existing in the knowledge of behaviour of the wilt fungus under certain physical conditions, the present studies were undertaken.

EXPERIMENTAL : Wilted linseed plants were collected from three different areas of the country *viz.*, Gurdaspur, Kanpur and Nagpur during 1955-57. Of the large number of isolations made, 10 isolates were selected for these studies.

Pathogenicity of these isolates was tested on four linseed varieties, namely, I.P.I.6, Hybrid 6, N.P. (R.R.) 9 and H. 614-1. I.P.I. 6 was found to be most susceptible and was, therefore, used as a control during the experimental work. The tests were carried out during November to March under glasshouse conditions. Wilting of seedlings was observed within three weeks after inoculation. All the isolates, except number 9, collected from Gurdaspur, proved pathogenic. Isolate Nos. 2, 4, 5, 6, 8 and 10 produced typical wilt symptoms throughout the period of investigation, while Nos. 1, 3 and 7 produced wilt symptoms only in the later part of the season when the soil temperature was high (25°-30°C), and isolate No. 6 proved to be the most virulent under all conditions.

(i) *Effect of soil moisture* : Four soil moisture levels ranging from 25 to 100 per cent were maintained. The tests were conducted in metal cans, 9 inches by 6 inches, having a side tube at about one inch from the base. Two-third of the can was filled with sterilised soil and then 100 gms. of the inoculum was mixed uniformly over this and covered with a sterilised soil layer of about one inch. Twenty seeds of linseed variety I.P.I. 6 were sown in each can.

The water holding capacity of the soil was determined and by adding varying amounts of water, the soil in the cans was adjusted to soil moisture of 25, 50, 75 and 100 per cent of water holding capacity. The cans were watered through the side tube and water was thus distributed evenly by capillary action. Suitable controls for each level of soil moisture were also maintained. The experimental cans were weighed twice daily and losses due to transpiration and evaporation were adjusted by adding the required amount of water.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Effect of soil moisture on the incidence of wilt

Percentage of soil moisture	Inoculated cans		Uninoculated cans		No. of plants wilted		Percentage of wilt	
	No. of seeds		No. of seeds		Inoculated cans	Uninoculated cans	Inoculated cans	Uninoculated cans
	Sown	Germinated	Sown	Germinated				
25.0	20	20	20	20	16	0	80.0	0
50.0	20	20	20	18	10	0	50.0	0
75.0	20	16	20	14	6	0	37.5	0
100.0	20	16	20	14	3	0	18.8	0

It would be observed from Table 1 that low moisture level was more favourable for disease development than high moisture level. The maximum wilt incidence (80 per cent) was obtained at 25 per cent saturation. The incidence of the disease gradually decreased with increasing saturations and it was only 18 per cent when the soil moisture level was 100 per cent. Verma (1945) has also reported that in case of linseed wilt percentage of infection was higher at 30 per cent than 40 per cent of soil moisture level.

(ii) *Effect of soil aeration* : Besides the unamended soil (loam) and sand, three different grades of soil having 25, 50 and 75 per cent sand were prepared for these studies. The soil as well as sand were sterilized before use. The fungus inoculum was added at the rate of about five per cent of the total quantity of the soil or sand in the pots. Eight seeds of I.P.I.6 variety were sown in each pot and the wilt incidence was recorded a month after sowing. The experiment was repeated twice. The results obtained are presented in Table 2.

TABLE 2 : Effect of soil aeration on the incidence of wilt

Treatment	No. of seeds		No. of plants wilted	Percentage of wilt
	Sown	Germinated		
Soil	16	15	6	40.0
25 per cent soil	16	14	13	92.8
50 per cent soil	16	16	12	75.0
75 per cent soil	16	14	3	21.4
Sand	16	15	12	80.0
Control (sand)	16	16	0	0
Control (soil)	16	16	0	0

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Maximum wilting (92.8 per cent, Table 2) occurred in the case of 75 per cent sand and 25 per cent soil mixture. Bolley and Manns (1932) pointed out that light-textured soils were more favourable for the development of various fungi associated with flax wilt than heavy-textured soils.

(iii) *Effect of addition of organic matter to soil* : Farmyard manure was added to the sterilised soil in the ratio of 1 : 20. The experiment was conducted in 6″ pots under glasshouse conditions. Suitable controls were also maintained. The results are presented in Table 3.

TABLE 3 : Effect of farmyard manure on the incidence of wilt

Isolate No.	Sterilised soil				Sterilised soil plus farmyard manure			
	No. of seeds		No. of plants wilted	Percentage of wilt	No. of seeds		No. of plants wilted	Percentage of wilt
	Sown	Germinated			Sown	Germinated		
1	10	8	4	50.0	10	9	4	40.4
2	10	8	5	62.5	10	10	6	60.0
3	10	10	1	10.0	10	8	0	0
4	10	9	4	40.4	10	10	3	30.0
5	10	8	4	50.0	10	10	4	40.0
6	10	10	10	100.0	10	10	9	90.0

It would be observed from Table 3 that addition of organic matter decreased the incidence of wilt.

Varietal resistance : Sixteen varieties of linseed obtained from Botany Division of this Institute, were tested for their resistance to the most pathogenic isolates of *Fusarium* under study. The experiment was conducted in 6″ pots under glasshouse conditions during January and February, 1959. At the time of inoculation, the top two inches of sterilized soil was removed from the pots and the fungus inoculum in the ratio of 1 : 20 was mixed in it. The soil was replaced over the inoculum and the pots were seeded with 8 seeds of the linseed variety to be tested. The seeds were later covered with sterilised soil. The results are presented in Table 4.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 4 : Reaction of 16 linseed varieties to the most virulent isolate (No.6) under glasshouse conditions

Sr. No.	Variety	Total No. of plants	No. of plants wilted	Percentage of wilt
1.	H.614-1	16	6	37.5
2.	I.P.I.6	13	8	61.5
3.	R.R.407	12	3	25.0
4.	R.R.439	16	3	18.7
5.	N.P.12	10	1	10.1
6.	N.P.124	8	2	25.0
7.	B.5128	15	1	6.6
8.	N.C.7.1	12	5	41.6
9.	Hybrid 8	15	5	33.3
10.	N.P. (R.R.)5	13	1	7.7
11.	N.P. (R.R.)9	14	2	14.4
12.	N.P. (R.R.)37	12	2	16.6
13.	N.P. (R.R.)38	8	2	25.0
14.	N.P. (R.R.)39	13	3	23.0
15.	N.P. (R.R.)45	6	2	33.3
16.	N.P. (R.R.)204	14	3	21.4

It would be seen (Table 4) that none of the 16 varieties tested showed complete resistance. However, the percentage of infection ranged from 6.6 to 61.5 in different varieties: the highest (61.5 per cent) being in the susceptible variety I.P.I.6 while 3 varieties, namely B. 5128, N.P. 12 and N.P. (R.R.)5, showed 6.6., 7.7 and 10.1 per cent infection, respectively, and may thus be considered resistant.

DISCUSSION : The results obtained show that the level of moisture in soil is an important factor in the development and incidence of the disease. At 25 per cent saturation, maximum wilt infection (80 per cent) was recorded, as compared to 18 per cent at 100 per cent soil-moisture level. It is very likely that high soil moisture affects the growth of the fungus by restricting the air supply. This also confirms, Vasudeva's report (1949) that soil-borne fungi in general, are affected by anaerobic conditions of soil which may result in poor, if at all, multiplication of fungi. The fact that soil aeration may have a direct influence on the growth and parasitism of the fungus was also borne out by the experiment wherein the soil was amended to different degrees of porosity by the addition of sand. A soil mixture having 75 per cent of sand and 25 per cent of loam soil, induced the maximum activity of the fungus (92.8 per cent wilting), while a 25 per cent sand and 75 per cent soil moisture produced only 21.4 per cent wilting.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SUMMARY

Low moisture and light sandy soils have been found to favour the development of the wilt fungus. Sixteen linseed varieties were tested against the most pathogenic isolate of the fungus. None of the varieties, however, showed complete resistance to the fungus.

ACKNOWLEDGEMENTS : The authors are extremely grateful to Dr. R. S. Vasudeva, former Head of the Division of Mycology and Plant Pathology, for his keen interest and valuable suggestions throughout the course of these investigations. Thanks are also due to Dr. B.L. Chona, Head of the Division of Mycology and Plant Pathology, for critically going through the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

BOZLEY, H.L. and T.F. MANNS, (1932) Fungi of Flax and Flax-sick soil. *Bull. N. Dak. Agric. Exp. Sta. No.* 259.

PEARL, R.T. (1923) Report of Mycologist to Government of C.P. and Berar. Rept. Dept, Agriculture, C.P. and Berar for the year 1924 : 19-20 (*Rev. Appl. Mycol.*, **3** : 77, 1924).

VASUDEVA, R.S. (1949) Soil-borne plant diseases and their control. (Presidential address), Section of Agriculture, 36th Indian Science Congress, Allahabad.

VERMA, A.R.S. (1945) *Fusarium* wilt of linseed. *Nagpur Agric. Coll. Mag.*, **19** : 12-15.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

NATURAL INFECTION OF OXALIS CORNICULATA L., THE ALTERNATE HOST OF PUCCINIA SORGHI SCHW., IN INDIA

D. P. MISRA AND S. K. SHARMA

(Accepted for publication May 12, 1964)

Some species of *Oxalis* including O. *corniculata* L., have been reported from various countries as alternate host of *Puccinia sorghi* Schw., and have been found to be responsible for the annual initiation of the disease on the crop. In India, natural infection of the rust on the species of *Oxalis* has not been reported so far, though under laboratory conditions Lele, Misra and Rao (1962) found *O. corniculata* to be susceptible to a maize rust collection from Simla. Recently Misra (1963) has reported *O. corniculata* to be the potential source for the annual recurrence of the maize rust in the hills of Nepal.

O. corniculata is a common perennial weed which grows upto 2, 500m. a.s.l altitude throughout the country (Collett, 1921). Maize is grown practically throughout the country mainly in the 'Kharif' season; however, its cultivation in the 'Rabi' season is also becoming popular. In the hills the crop extends to about 3,700m. a.s.l. and is sown in about May-June normally, but early sowings from March onward are not uncommon to suit local requirements.

P. sorghi has been reported from various regions of the country and particularly in hilly regions of India it causes considerable losses. In the hills of Northern part of the country the rust is reported to damage the crop in the seedling stage itself*. However, regarding the modes of annual recurrence of the disease, little is known. With the finding of the role of the alternate host in the annual initiation of the disease in Nepal, the possibilities of similar situation in India were indicated. The present paper deals with the role of teliospores of the rust in relation to its recurrence in the hills and subsequently, its potential danger to the 'Rabi' crop in the plains.

OBSERVATIONS AND EXPERIMENTS : Pycnial and aecial stages on *Oxalis corniculata* L., were found in the vicinity of Simla from a few places at approximate altitudes from 1, 700 to 2, 250m. a.s.l., during the months of April-June 1963. The aeciospores on inoculation produced uredia on maize seedlings in about 10 days at average range of temperatures of 10-20°C., and the morphological characters of these stages of rust were found to agree with *Puccinia sorghi*.

Near the infected *O. corniculata* plants, straw of maize bearing telia was invariably found. This telial material and other similar overwintered telial collections in nature, and those stored under a shed and

*Annual Roport of 'Indian Agriculture Program' by the Rockefeller Foundation, Agric. Sec., 1959-60.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

kept in room at Simla were tested for germination. The results given in the table show that the teliospores exposed to natural conditions after the harvest of maize crop in October readily germinate at 12-18°C., in case humidity is available even for a few hours and these are viable in nature upto the middle of June at least. The teliospores stored in room and under a shed at Simla germinate in 7-10 days when adequate humidity and temperatures are made available.

In another set of experiments it has been found that the readily germinable teliospores do not garminate at 2-9°C even in 15 days but start normal germination when transferred to optimum temperatures of 12-18°C. Similarly, the spores from this material do not germinate at temperatures higher than 23°C. The teliospores floated in tap water for 3 days at 27-28°C show only 5 per cent germination when transferred to optimum temperatures and the spores completely lose their viability at 30-31°C when floated in tap water for three days.

It has been found that the germinating teliospores can cause infection on *O. corniculata* even if adequate humidity is provided for 3-6 hours at 15-20°C.

Earliest appearance of uredial stage on maize crop has been found in the beginning of June at Simla.

DISCUSSION : The teliospores of *P. sorghi* are found to be killed at 27-31°C when floated in water for a few days only. Such temperatures or even much higher temperatures are prevalent in the plains and in lower hills for a considerable period between the harvest of maize crop and its next sowing season. Therefore, the teliospores in such areas can be hardly of any consequence, in infecting the alternate host, *O. corniculata*, though this weed is common there. On the other hand the teliospores have been found to be dormant at temperatures below 9°C. and such lower temperatures exist for most of the time in the diurnal cycle during the months of November-March, in the North Indian Hills above 1,700m. a.s.l. The teliospores exposed to such conditions readily germinate under congenial conditions that are available from about April onwards and cause infection on the alternate host, which occurs upto 2, 500 m. a. s. l. The continued cycle of aecial infections upto even June is potential enough to cause initial uredial infection on maize crop and this has actually been found in early June, in nature at Simla. Thus the initiation of the disease through sexual phase of the rust involves the possibility of occurrence of large number of physiologic races of the rust in the country.

It does not seem possible to eradicate a common weed like *O. corniculata*. However, if the straw of maize is destroyed after the harvest in the hills, this may reduce the potentialities of initial infection on the alternate host. But even to destroy all telia-bearing maize straw in the hills is by no means an easy task, year after year. Therefore, it may be necessary to determine the pathogenicity of rust on maize varieties and evolve resistant material for cultivation in the hills to start with.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Collections of teliospores from maize in vicinity of Simla during 1962-1963, their treatment and gremination

S. No.	Place and date of collection, its storage, etc.	Further treatment	Percentage of germination	Remarks
1.	From Simla (2,150m. a. s. l.) during October 1962			
	(i) stored in room at Simla from Oct., 1962 to 13/4/1963	(a) spores floated* in tap water for 7 days	10-40	
		(b) spores floated* in tap water after 3 alternate wettings** and drying***, each for 2 days	Above 60	
	(ii) Stored under a tin shed at Simla from October, 1962 to 13/4/1963	(a) spores floated* in tap water for 7 days	10-40	
		(b) spores floated in tap water after 3 alternate wetting** and dryings***, each for 2 days	Above 60	
	(iii) Exposed to natural conditions at Simla			
	(a) from October, 62 to 10/4/1963	spores floated* in tap water for one day	Above 70	Some spores started germinating in 2-6 hours
	(b) from Oct., 62 to 23/4/1963	—do—	—do—	
	(c) from Oct., 62 to 3/5/1963	—do—	Above 60	
	(d) from Oct., 62 to 13/5/1963	—do—	—do—	
	(e) from Oct., 62 to 23/5/1963	—do—	Above 50	
	(f) from Oct., 62 to 2/6/1963	—do—	40-50	
	(g) from Oct., 62 to 12/6/1963	—do—	About 30	Tests discontinued afterwards
2.	From Kail village (1,700m.a.s.l.) in nature on 12/4/1963	—do—	Above 60	

*At 12-18°C.; **At 4-7°C.; ***At 15-18°C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

So far, maize rust has not been a serious disease in the plains of India, where the crop is mainly cultivated in the 'Kharif' season and the temperatures are usually higher than 28°C. This may be so, because the uredospores are unable to cause infection at 28-32°C. and the normal infection is produced at 18-20°C. (Weber, 1922). However, if the cultivation of maize during the 'Rabi' in India becomes common, there are chances of enormous losses due to rust on account of the disease in uredial stage in the hills during the summers and in the plains during the winters. Therefore, it appears necessary to study the race flora of the rust in the country and get ready with the resistant varieties in advance for the 'Rabi' maize crop for the plains.

Summary

Natural infection of *Puccinia sorghi* on *Oxalis corniculata* has been found from April-June in Simla hills. Teliospores on straw remain viable in the hills above 1,700m. a.s.l. For the annual initiation of the rust, the alternate host is a potential source. Future problems that are likely to arise are discussed.

Acknowledgements : The authors are indebted to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, for the encouragement during the course of these studies. Grateful thanks are also due to Dr. R. Prasada, Mycologist for kindly going through the manuscript and making useful suggestions by which the utility of the paper has greatly improved.

Indian Agricultural Research Institute,
Plant Pathological Research Sub-station,
Flowerdale, Simla.

References

Collett. H. (1921) *Flora Simlensis*, Thacker, Spink & Co.

Lele, V. C., D. P. Misra, & M. H. Rao, (1962) Aecial stage of *Puccinia sorghi* Schw., the rust of maize, in India. *Indian Phytopath.*, **15** : 164-165.

Misra, D. P. (1963) Natural occurrence of the aecial stage of *Puccinia sorghi* Schw. on *Oxalis corniculata* Linn. in Nepal. *Indian Phytopath.*, **16** : 8-9.

Weber, G. F. (1922) Studies in the corn rust. *Phytopathology*, **12** : 89-97.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FURTHER STUDIES IN THE CROWN RUST OF OAT IN INDIA

D. P. MISRA, S. K. SHARMA, P. C. JOSHI AND SHEODHAN SINGH

(Accepted for publication February 24, 1964)

Puccinia coronata var. *avenae* Eriks., is not an important pathogen in India so far, although it has been reported to occur in the eastern region of the country. It, however has been reported to be a serious disease in temperate, humid and semi-humid regions of various countries. (Simons and Murphy, 1955; Waterhouse, 1952; Straib, 1937; and Vallega. 1942). The Punjab local oat (*Avena byzantina*) has been found to be resistant to races 231 and 240 of crown rust of oat reported earlier from India (Payak and Misra, 1963) and most of the local varieties belong to this species. However, recent trend of using oats as breakfast food has resulted in the introduction of certain foreign improved oat varieties in the country. These introductions have been made from the countries where crown rust is an important disease and it is likely to pose a new problem before us. Therefore, attempts were made to determine the physiologic races present in the country. Certain grasses and varieties of Oat were tested with the available races in order to get information on the collateral hosts and performance of the varieties.

New race of crown rust of oat : A collection of crown rust of oats from Uttar Pradesh (crop of 1962-63) when tested on differential varieties (Simonds and Murphy, 1955), reacted similar to race 227 as shown in Table 1.

TABLE 1 : Reactions* of a new race of *Puccinia coronata* var. *avenae* and race 227 from U. S. A. on differential varieties

Stock culture or isolation	Anthony	Victoria	Appler	Bond	Landhafer	Santa Fe	Ukraine	Trispernia	Bondwic	Saia	Victory as Control
Sitapur Oat Var. ? and S. S. culture	S	R	S	R	R	I	S	R	I	S	S
Race 227	S	R	S	R	R	R	S	R	R	S	—

*S-Susceptible ; I-intermediate ; R-Resistant.

The new race resembles in its reactions on most of the differential varieties with race 227, except that it produces Intermediate type of infection on Santa Fe and Bondwic which are resistant to race 227. In spite of these minor differences which may be due to environment, this race is designated as race 227.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Varietal Resistance Tests : Eighteen varieties of oats were tested with races 231, 240 and 227 individually and their reactions are presented in Table 2.

TABLE 2 : Reactions* of 3 physiologic races of crown rust on 18 varieties of oats

Oat variety	Accession nos.	Reactions produced by races		
		231	240	227
Overland	E. C. 5678	R	R	R
Boris opus	E. C. 2283	S	S	S
Kent	E. C. 13594	S	S	S
Adliker	E. C. 17887	S	S	S
White Algerian	E. C. 3230	S	I	S
Dale	—	S	S	S
Ballidue		S	S	S
Flaming Gold	E. C. 4262	S	S	S
Avon		S	S	S
New Australian	E. C. 21278	R	R	I
Curt	E. C. 22023	S	S	S
Richland		S	S	S
Joanette		S	S	S
Minrus		S	S	S
Hyb. X-27		I	I	I
Hyb. 3		S	S	S
Victory		S	S	S
Punjab local (*A. byzantina* variety)		2/15 13/15	2/16 14/16	
		S R	S R	I

*S-Susceptible ; I-intermediate ; R-Resistant.

It would be seen from Table II, that only one variety Overland, is resistant to all the three races found in India.

Collateral host studies : Role of collateral hosts in the perpetuation and spread of rusts is well known in various countries of the world. In India, Payak and Misra (1963) have reported *Avena fatua* to be susceptible to races 231 and 240. Seventeen species of genera *Avena*, *Andropogon*, *Bromus*, *Hordeum*, *Phalaris* and *Vulpia* were tested with the 3 races of crown rust individually. The results show that *Avena glauca* and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phalaris minor are susceptible to all the three races. *P. tuberosa*, gets infected with race 231, *A. strigosa* with race 240 and *A. sativa* and *Vulpia myuros* with race 227.

Other thirteen species namely *Aegilops crassa* (E. C. 12039), *A. squarrosa* (E. C. 13247), *Arrhenatherum elatius* (E. C. 17335), *Bromus catharticus* (I. W. 65), *B. japonicus* (I. W. 78), *Dactylis glomerata*, *D. tarrus*, *Festuca pratensis* (E. C. 16733), *Hordeum murinum* (E. C. 2313), *Lolium perenne* (E. C. 16743), *Phalaris* (from Nepal), *Phleum pratens* (E. C. 16734), *Secale cereale* (Russian Rye) were tested with a mixture of the three races and were found resistant.

DISCUSSION: A race of Crown rust of Oats reported herein resembles race 227. This race appears to be more virulent than the earlier reported races 231 and 240 (Payak and Misra, 1963) in that it infects oat varieties Appler, Saia, *Avena sativa* (Australian) and *Vulpia myuros*. It also infects Punjab local oat a variety of *Avena byzantina* to which most of the cultivated local oats belong and therefore, seems to be an important race for this country.

Out of 18 oat varieties tested against 3 physiologic races of crown rust, only Overland, a variety from U. S. A., is resistant which may be used as resistant donor in breeding crown rust resistant varieties of oats. This variety appears to have an ample scope for utilisation in improving the existing varieties, because most of them are susceptible to this rust. In case, susceptible varieties which may be otherewise good for cultivation in the country are grown extensively, these are likely to suffer losses. Therefore, crown rust resistance should be incorporated in the varieties that are released for cultivation.

Mode of perpetuation of crown rust of oat in India, is not definitely known. However, the role of both, the alternate host *i. e.* susceptible species of *Rhamnus* in the hills as well as of collateral hosts can not be ruled out. Species of *Rhamnus*, susceptible to this rust and their distribution has not been determined so far. But the collateral host studies indicate the possibility of perpetuation of this rust on certain indigenous grasses, *viz. Avena fatua* (Payak and Misra, 1963), *Phalaris minor*, *Vulpia myuros* and exotics *viz. Avena strigosa* and *Phalaris tuberosa* which have been found to be susceptible to crown rust of oats under laboratory condition, although natural infection of this rust on these grasses has not been found.

SUMMARY

A race of *Puccinia coronata* var. *avenae* Eriks resembling race 227 has been identified from India. Out of 18 varieties, Overland an exotic variety, was found resistant to the 3 Indian races of crown rust. *Phalaris minor*, *Avena glauca A. strigosa*, *Phalaris tuberosa* and *Vulpia myuros* are susceptible.

ACKNOWLEDGEMENTS: Authors are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, for his encouragement during the course of these studies. Thanks are also due to Dr. R. Prasada for making useful suggessions in the manuscript. Receipt of seeds of most of the varieties of oat and grasses from Shri H. B. Singh,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Head of the Division of Plant Introduction, I. A. R. I., is gratefully acknowledged.

Indian Agricultural Research Insititute,
Plant Pathological Research Sub-Station,
Flowerdale, Simla-E.

REFERENCES

PAYAK, M. M. AND D. P. MISRA, (1963) Physiologic specialisation in *Puccinia coronata* Corda. in India. *Indian Phytopath.*, **16** : 15-20.

SIMONS, M. D. AND H. C. MURPHY, (1955) A comparision of certain combinations of oat varieties as crown rust differentials *Tech. Bull.* No. 1112, U. S. D. A.

STRAIB, W. (1937) Die bestimmung der physiologischen rassen von *Puccinia coronata* CDA suf hafer in Deutschland. *Biol. Reichoanst f. land U. Forstw. Arh.* **22** : (121)-157.

VALLEGA, J. (1942) Especializacion fisiologica de *Puccinia coronota avenae*, en Argentina, *Santa Catalina Inst. Fitotec. An.* (1940) **2** : 53-82.

WATERHOUSE, W. L, (1952) Australian rust studies, IX Physiologic race determinations and surveys of cereal rusts. *Proc. Linn. soc. N. S. Wales*, 77 : 209-258.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RAMULARIA AREOLA ATK. ON HERBACEUM COTTONS IN VIDHARBHA (MAHARASHTRA)

M. R. SIDDIQUI AND S. B. P. RAO

(Accepted for publication July 30, 1964)

Ramularia areola Atk. has been reported as a minor disease of cotton in the United States, South Africa, Brazil, Egypt and Middle East. As regards India, our knowledge regarding its occurrence and spread is quite meagre. Uppal (1948) reported this disease as only of minor importance and not serious enough to warrant any control measures. It has, however, been reported from Madras, Old Bombay State and Madhya Pradesh. Further, in the past few years, this disease has been assuming serious proportions in Vidharbha on *arboreum* cottons.

Based on the cotton acerage, and the species grown, India has been divided into different regions, one such region is designated as 'Central-*arboreum*-Zone', because of the predominance of *Gossypium arboreum* covering 47 lakhs acres out of 64 lakhs under cotton cultivated in Maharastra. Vidharbha plays an important role in the region. This area was badly hit due to the increasing incidence of grey mildew disease. Disease resistance is of paramount importance for obtaining good yields now.

The disease is known to be limited to ('*deshi*') *arboreum* cottons. But during 1963-64 one isolated field of *herbaceum* cotton showed a heavy infection of grey-mildew. This was spotted in a field situated about three miles away from Amravati town. Another interesting fact worthy of note was that the *arboreum* fields, in the vicinity showed very little or no infection at all. Recently, in the PIRRCOM fields, infection of grey-mildew was also noted on four *herbaceum* varieties (*viz.*, Vijalpa, Suyog, Wagad-8, and 1027-A.L.F.). Collections were made to study variations, if any, in this fungus, in comparision to those reported on *arboreum* cottons earlier. The preliminary findings are reported briefly in this note.

SYMPTOMS : The disease is locally known as "dahiya", due to the white-frosty growth of the fungus, mostly seen on the lower surface of the leaves. The disease appeared in the month of October as irregular, angular, pale, transluscent-spots, measuring 2-4 mm. The number of spots per leaf increased in the first week of November and were more white in colour, than those found on *arboreum* cotton. The frosty growth consisting of conidia and conidiophores of the fungus appeared mainly on the lower surface. But a good number of leaves showed the infection on both sides of the lamina. With the increase of infection, the affected leaves became yellowish-brown and finally dropped down. Another, important difference noted on diseased *herbaceum* cotton was, that many green leaves remained on the plants, unlike those of affected *arboreum* plants, where almost all the leaves are shed.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

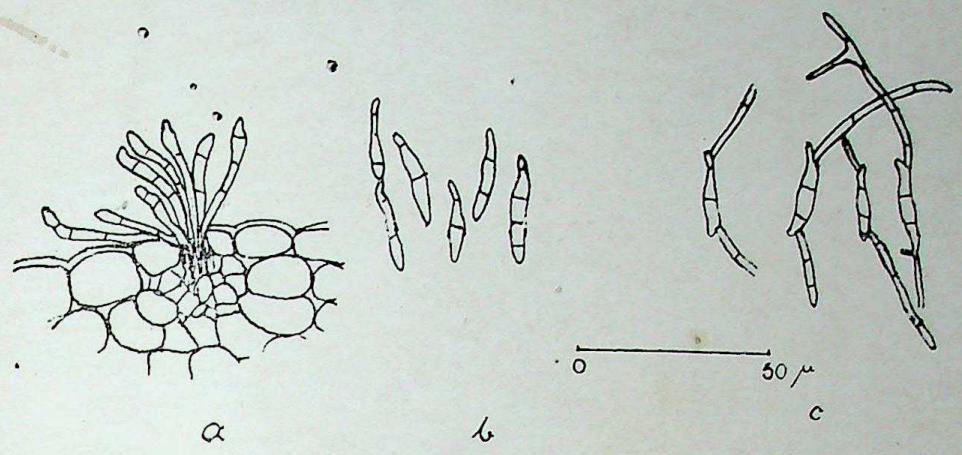


Fig. 1. *Ramulara areola* : a. Section showing the conidiophores. b. Conidia. c. Germinating conidia.

ETIOLOGY : Transverse sections of the infected leaves showed that the mycelium was restricted to the disease lesions. The condiophores as shown in Fig. 1(a) emerge in hyaline clusters of parallel hyphae, with 2-3 septa, rounded or flattened at the ends, and measuring 25-70×4.5-6μ. Conidia are formed in chains of 2-4 spores acropetally at the tips, and the lateral projections of the conidiophores. The chains break off soon; and the conidia appear singly. These are colourless, irregularly oblong, with abruptly pointed ends but sometimes with rounded or flattened ends. They are 1-3 septate, measuring 15-40×3-4.5 μ (Fig. 1(b).

Cultural and Pathogenic Studies : Conidial germination occurred on 2 per cent sugar solution. 40-50 per cent germination was obtained in 12-18 hours at room temperature (20-22°C). Germ tubes arise from either one or both the ends. (Fig. 1(c)). The fungus has been brought in culture on cotton juice-dextrose agar medium.

Artificial infections were also carried out following the direct leaf-attachment method. Cotton seedlings (*arboreum*) raised in the pots as small plants of *herbaceum* varieties in the field were watered well, and covered with polythene tubes. Seedlings and the tubes were sprayed with water before covering the seedlings with the polythene-tube. At about 3 P.M., infected *herbaceum* leaves were fixed on to the lower surface of the young leaves of *arboreum* plants with a wet wad of cotton. Copper rings were put on the cotton wad, in order to hold the leaves firmly. The seedlings were watered regularly, and the polythene tubes were sprayed from outside, to raise the humidity. Inoculated seedlings were kept inside a humidity tent. Disease symptoms were clearly seen on the *arboreum* seedlings kept inside the humidity tent after 10-12 days; while inoculations made in the open field proved to be negative. It is thus clear that both high humidity as well as low temperature play an important role in causing the infection.

So far as the authors are aware, this disease is being noted for the first time on *herbaceum* cottons in India. Representative specimens are being deposited at the *Herb. Crypt. Indiae Orient.*, New Delhi. This

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

study was undertaken in the PIRRCOM project under the aegis of I.C.A.R., New Delhi.

Regional Research Centre,
PIRRCOM, Amaravati,
Maharashtra.

References

BLANK, L.M. (1953) *The Year Book of Agriculture*, U.S. Dept., Agric.

BUTLER, E.J. (1918) *Fungi and disease in plants*. Thacker Spink and Co., Calcutta.

KHABIRI, E. (1952) Contribution a la mycoflore de l' Iran Premiere liste (A contribution to the mycoflora Iran. First list) *Rev. Mycol.* **17**.

LITZENBERGER, S.C. AND J.A. STEVENSON (1957) A preliminary list of Nicarguan plant diseases. *Plant dis. Reptr.* Suppl. 243.

UPPAL, B.N. (1948) *Diseases of Cotton in India*, I.C.C.C., Bombay.

VASUDEVA, R.S. (1960) *Cotton in India*, **2** : 189-190.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THREE NEW BACTERIAL DISEASES OF PLANTS FROM MAHARASHTRA[1]

L. MONIZ[2] AND V. P. BHIDE[3]

(Accepted for publication July 15, 1964)

INTRODUCTION : During the rainy season of 1955, diseased specimens of onion (*Allium cepa* L.), *Martynia diandra* Glox. and *Vitis carnosa* Wall. received from Nasik and Jalgaon districts showed infection by phytopathogenic bacteria. As all the three diseases appeared to be new records, an investigation of their causal organisms was undertaken and a preliminary note was published (Moniz and Patel, 1958); the present paper is a more detailed account of the investigation.

MATERIAL AND METHODS : Preparation of media, staining procedures and study of cultural and biochemical characters of the bacteria were carried out as per standard methods (Manual of Microbiological Methods, 1957). Seedlings of the various hosts for infection experiments were raised from surface-sterilised seeds planted in steamed soil in earthen pots kept in a glasshouse at Poona where the air temperature fluctuated from 25° to 30C° during the period of tests. Ridgway's (1912) colour standards were used for describing colours of colonies of the organisms.

(1) Seedling blight of onion (*Allium cepa*)

A bacterial disease of onion seedlings was first reported from Colorado, U. S. A. in 1948 but was not fully investigated; streaks on leaves and premature death were the main symptoms.

Later, Burkholder (1942, 1950, 1954) described *Phytomonas alliicola* and *Pseudomonas cepacia* on onion and also found *Ps. marginalis* and *Ps. cichorii* from lettuce to be pathogenic to onion bulbs in infection experiments. Volcano (1954) described a variety of *Ps. syringae* causing a leaf disease of onion in Israel and Okabe *et al.*, (1956) found *Ps. destructans* to be a weak strain of *Ps. marginalis* from onion.

The disease in Nasik district of Maharashtra State is characterised by drying of leaves from tips downwards and in severe cases plants are killed outright. Microscopic examination of sections of diseased leaves shows bacteria oozing out in large numbers from the cut ends.

The organism was isolated in pure culture by the usual pour-plate method using potato dextrose agar. Pathogenicity of the organism was proved by spraying a watery suspension of the organism on young onion seedlings in pots kept under moist chambers. Blighting of leaf tips

---

[1]Taken from the thesis submitted by the senior author to the University of Poona, Poona for the Ph. D. (Agri) degree under the guidance of the junior author.

[2] and [3] respectively, Associate Professor of Agricultural Bacteriology, College of Agriculture, Poona and Agricultural Bacteriologist, Biological Nitrogen Fixation Scheme, College of Agriculture, Poona.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

started 2-3 days after inoculation and progressed rapidly thereafter. The organism was reisolated from such diseased leaves.

Morphological, Cultural and Physiological Characters: The organism is a short rod with rounded ends, single, and measures 1.6 (1.1-2.0) × 0.9 (0.7-1.1) microns. Each cell has 1-2 flagella at one end. The organism is Gram-negative, capsulated, non spore-former and non acid-fast.

Colonies on potato dextrose agar are circular with entire margins, depressed centres, pearly white, shining and butyrous; similar growth on potato cylinders and yeast glucose chalk agar; potato cylinders turned dark grey in 5 days. Poor growth on Czapek's agar and in nutrient broth, good growth on Patel's agar and Clara's medium (no fluorescence), Koser's uric acid medium and Simmon's citrate agar.

Litmus milk is slowly turned alkaline. Gelatin is not liquefied (Frazier's medium). Starch not hydrolysed. Casein is digested. Hydrogen sulphide and ammonia produced but not indole. Nitrate reduced beyond nitrite stage. Methyl Red and Voges-Proskauer tests negative. Acid but no gas from dextrose, lactose, xylose, raffinose, levulose, mannitol, glycerol, dulcitol, salicin and dextrin; no acid from sucrose and galactose. Ammonium citrate, ammonium phosphate, ammonium sulphate and ammonium tartrate utilised but not ammonium, calcium, magnesium, sodium and barium nitrates as sole source of nitrogen. Good growth on methionine, aspartic acid, creatine, glutamic acid, norleucine, guanidine hydrochloride, serine, tryptophane and tyrosine used singly as nitrogen source in a basal salts medium with sucrose as energy source. No browning of tyrosine crystals. Sodium chloride tolerated upto 4 per cent in a liquid medium. Optimum temperature for growth in culture 25° to 31°C.; thermal death point about 61°C. Obligate aerobe. Optimum H-ion concentration for growth in culture is 6 to 8.

Host range: Healthy seedlings of the following plants were sprayed with a watery suspension of the organism to find out its ability to infect other hosts besides onion: *Gloriosa superba* L., *Dipcadi montanum* Baker, *Canna indica* L., *Zephyranthes grandiflora* Lindl., *Polianthes tuberosa* L., *Asphodelus tenuifolius* Cav., *Curcuma longa* L., *Lycopersicon esculentum* Mill, *Citrus sinensis* Osbeck, *Allium sativum* L. and *A. cepa* L. (control). The organism could infect garlic, besides onion but not any of the other hosts tested.

Varietal resistance: The following varieties of onion, obtained from the Agricultural Research Station, Niphad, Nasik district were tested for their reaction to the organism in an infection experiment in a glasshouse: 118-46-TTS, 339-IS, 5-8-2, 2-4-ITS, 53-IS, 49-IS, and 404-IS. Only two varieties, *viz.*, 5-8-2 and 53-IS showed some degree of resistance the rest being highly susceptible.

Identity of the pathogen:— Moniz and Patel (1958) named the organism *Phytobacterium siccatum*. However, the genus *Phytobacterium* has not been recognized in Bergey's Manual (1957); it is, therefore, considered desirable to retain the generic name *Pseudomonas* for this organism and the binomial *Pseudomonas siccata* (Moniz and Patel)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Moniz and Bhide comb. nov. is proposed for it.

(2) Angular leaf spot of *Martynia diandra*

The disease on this common weed is characterised by the appearance of minute, angular spots on lower surface of leaves; these spots enlarge, coalesce and form irregular patches of light brown dead tissue later in the season.

The organism was isolated in pure culture by the pour-plate method and its pathogenicity proved on seedlings of *M. diandra* by inoculation experiments in a glasshouse.

Morphological, Cultural and Physiological characters : The organism is a short rod with rounded ends, single and measures 1.3 (0.83-1.58) × 0.68 (0.37-1.08) microns. The organism is monotrichous, Gram-negative, non spore-former and non acid-fast.

Colonies on potato dextrose agar are circular with entire margins, glistening, convex, Baryta yellow; similar growth on potato plugs and nutrient agar. Poor growth on Czapek's agar, Simmon's citrate agar and Clara's medium.

Litmus is completely reduced in litmus milk. Gelatin liquefied (Frazier's medium). Starch hydrolysed and casein digested. Hydrogen sulphide produced but not indole. Nitrate not reduced. M. R. V. P. tests negative. Acid but no gas from dextrose, levulose, lactose, xylose, galactose, sucrose, mannitol and glycerol; no growth in salicin; no change in reaction with raffinose and dulcitol. Ammonium citrate, ammonium nitrate, ammonium phosphate, ammonium sulphate, calcium nitrate, potassium nitrate and sodium nitrate utilised as source of nitrogen but not barium nitrate. Good growth with methionine, asparagine, aspartic acid, glutamic acid, norleucine, guanidine hydrochloride and tyrosine used singly in a basal inorganic salts medium with sucrose as energy source. Sodium chloride tolerated upto 3 per cent in a liquid medium. Optimum temperature range for growth in culture is 27° to 30°C. Thermal Death point about 52°C. Obligate aerobe. Optimum H-ion concentration for growth in culture 5.2 to 7.4.

Host range : In inoculation experiments to determine the host range of the pathogen, infection was produced only on *Martynia diandra* and *Sesamum indicum* L. but not on *Ipomoea muricata* Jacq.; *Vitis carnosa* Wall., *Phaseolus vulgaris* L., *P. lunatus* L., *Vicia faba* L., *Pisum sativum* L., *Dolichos lablab* L., *Vigna catjang* Endl., *Cicer arietinum* L., *Lathyrus sativus* L., *Arachis hypogaea* L., *Crotalaria juncea* L., *Medicago sativa* L., *Glycine max* Merrill, *Triticum vulgare* Vill., *Gossypium herbaceum* L., and *Solanum tuberosum* L.

Identity of the pathogen : The morphological, cultural and physiological characters of the pathogen show that it is a species of *Xanthomonas*. Only one bacterial plant pathogen, *viz.*, *Pseudomonas martyniae* (Elliott) Stapp has been described on *Martynia louisiana*. Since the organism reported herein belongs to another genus it is designated as a new species, *viz.*, *Xanthomonas martynicola* (Moniz and Patel, 1958).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(3) Leaf spot of *Vitis carnosa*.

This disease is characterised by the appearance of minute, water-soaked spots on the lower surface of leaves. The spots later turn dark, rough and are surrounded by yellow 'halos'. Centres of spots are raised due to deposition of dried bacterial exudate. Only leaves are affected.

The organism was isolated in pure culture and its pathogenicity to seedlings of *V. carnosa* was established by inoculation experiments.

Morphological, Cultural and Physiological characters of the pathogen : The organism is a short rod with rounded ends, single and measures 2.0 (1.47-2.48) × 0.9 (0.73-1.4) microns. It is monotrichous, Gram-negative, non-sporulating, capsulated and non spore-former.

Colonies on potato dextrose agar are circular with entire margins, Barium yellow, glistening and convex. Similar growth on potato plugs and nutrient agar. It grows well on Czapek's agar, Patel's agar, Clara's medium and nutrient broth, no growth on Koser's uric acid medium and on Simmon's citrate agar.

Litmus in litmus milk is completely reduced. Gelatin is liquefied (Frazier's medium). Starch hydrolysed and casein digested. Ammonia and hydrogen sulphide produced but not indole.

Nitrate not reduced. M. R. and V. P. tests negative. Acid, but no gas, from dextrose, xylose, lactose, sucrose, levulose, galactose, glycerol and mannitol; no change in reaction with raffinose and dulcitol; salicin not utilised. Ammonium citrate, ammonium nitrate, ammonium sulphate, ammonium tartrate, calcium nitrate, potassium nitrate utilised but not barium nitrate. Good growth with methionine, asparagine, aspartic acid, glutamic acid, norleucine, guanidine hydrochloride and tyrosine used singly in a basal salts medium with sucrose as energy source. Sodium chloride tolerated upto 3 per cent in a liquid medium. Optimum temperature for growth in culture is 27° to 31°C.; thermal death point about 51°C. Obligate aerobe. Optimum H-ion concentration for growth in culture is 5.2 to 7.3.

Host range: In inoculation experiments to determine the host range of the organism only *Vitis carnosa* was successfully inoculated but not any of the following plants : *Vitis vinifera* L., *Phaseolus vulgaris* L., *P. lunatus* L., *P. aureus* Ham., *Vicia faba* L., *Pisum sativum* L., *Dolichos lablab* L., *Cajanus cajan* (L) Millsp., *Vigna catjang* Endl., *V. sinensis* Endl., *Cicer arietinum* L., *Lathyrus sativus* L., *Arachis hypogaea* L., *Crotalaria juncea* L., *Medicago sativa* L., *Glycine max* Merrill, *Cyamopsis tetragonoloba* (L.) Taub., *Triticum vulgare* L., *Lycopersicon esculentum* L., *Gossypium arboreum* L., *G. herbaceum* L., and *Solanum tuberosum* L.

Identity of the pathogen: The organism is evidently a new species of *Xanthomons* in view of its characters and was so described, [X. *vitis-carnosae* by Moniz and Patel (1958) (Sic)].

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The authors are grateful to Dr. M. K. Patel, retired Plant Pathologist to Government, B. S., Poona for help and guidance in this investigation.

Plant Pathological Section,
College of Agriculture,
Poona.

REFERENCES

ANONYMOUS (1952) A new onion disease. *Seed World* **71** : 25.

BREED, R. S., E. G. D. MURRAY, AND N. R. SMITH, (1957) *Bergey's Manual of Determinative Bacteriology*. 7th ed. Williams and Wilkins Co., Baltimore, U. S. A.

BURKHOLDER, W. H. (1942) Three bacterial plant pathogens. *Phytopathology*, **32** : 141-143.

——————— (1950) Sour skin, a bacterial rot of onion bulb. *Phytopathology*, **40** : 115-117.

——————— (1954) Three bacteria pathogenic on head lettuce in New York. *Phytopathology*, **44** : 592-596.

MONIZ, L. AND M. K. PATEL (1958) Three new bacterial diseases of plants from Bombay State. *Curr. Sci.*, **27** : 494-495.

OKABE, N. AND M. GATO (1956). Comparative studies on the pathogenic, physiological and cultural characters of Pseudomonas spp. *Bull. Faculty Agri. Schiznoka Uni.*, **6** : 5-15.

RIDGWAY, R. (1912). *Colour standards and colour nomenclature.* Washington, D. C. (Published by the author).

SOCIETY OF AMERICAN BACTERIOLOGISTS (1957) *Manual of Microbiological Methods.* McGraw-Hill Book Co. New York, U. S. A.

VOLCANO, Z. (1954) An onion and tomato disease caused by a variety of *Pseudomonas syringae*. *Bull. Res. Corr. Israel*, **4** : 171-175.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EVALUATION OF CERTAIN FUNGICIDES FOR THE CONTROL OF RHIZOCTONIA BATATICOLA (TAUB.) BUTLER AND SCLEROTIUM ROLFSII SACC.

DHARAM VIR, K. D. PATHAK AND S. B. MATHUR

(Accepted for publication on July 4, 1964)

A number of proprietary fungicides are being marketed and a few of these are being used as soil fungicides for the control of some of the soil-borne diseases of different crops. The relative efficacy of a large number of fungicides can not be assessed in the field owing to the limited space and other facilities available at different Research Stations and such a type of test also requires a fairly long period before conclusive results can be obtained. A laboratory technique which has been successfully devised by Zentmyer[1] was followed to test the relative efficacy of a few fungicides against *Rhizoctonia bataticola* and *Sclerotium rolfsii* which are the common soil-borne fungi and have a fairly wide host range. This technique is reported to have given a close correlation between laboratory and field performance of fungicides when applied to soil.

EXPERIMENTAL : Fine loamy sandy soil collected from the field was airdried and autoclaved for 45 minutes at a pressure of 15 lbs./sq. inch and 21.0 gms. of such soil was placed in sterilised specimen tubes of 3″ × 0.7″. The test fungi were grown on* potato dextrose agar and agar discs, 9 mm. in diameter, were cut in the case of *Rhizoctonia bataticola* after a growth period of a fortnight. The disc was placed in the specimen tube at a depth of one inch from the top surface layer of the soil. In the case of *Sclerotium rolfsii*, single sclerotium of uniform size was used instead of a disc, for different treatments. Five ml. of the fungicidal solution to be tested was applied to the surface of the soil in the specimen tube with the help of a pipette. This amount of solution was found to be sufficient to just wet the whole of the soil. The solutions of various fungicides were prepared in sterilised distilled water. In the case of the controls, only sterilised distilled water was applied to the soil. These specimen tubes were then incubated at 25°C for 24 hours. For each of the treatment, five replications were kept. After the incubation period, disc or the sclerotium from each tube was removed with the help of sterilised foreceps, washed in sterilised water and then plated on potato dextrose agar in petri dishes in order to determine the viability of the fungus. The data has been recorded for fungicidal properties of the chemicals e.g. on the basis of presence or absence of the growth of the fungus on the medium. A large number of fungicides representing different groups were tested. In the first instance, all the fungicides were screened at 0.2 per cent concentration (*i. e.*, 2000 ppm. of fungicide solution in water) and those which did not show any encouraging results were discarded from further tests. In

[1]Zentmeyer, G.A. *Phytopathology*, 45 : 398-404, (1955).

*Peeled potatoes 200 gms; dextrose 20 gms; agar agar 20 gms; Distilled water 1000 cc.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE : Comparative efficacy of different fungicides against *Rhizoctonia bataticola* and *Sclerotium rolfsii*

Proprietary name of fungicide	Chemical name (Active ingredient)	Dose %	Mycelial growth on PotatoDextrose agar-Five replications	
			Rhizoctonia bataticola	*Sclerotium rolfsii*
Fytolan	Copper oxychloride (50 per cent copper)	0.2	+	+
Shell copper fungicide	Copper oxychloride -do-	0.2	+	+
Copper sandoz	Cuprous oxide -do-	0.2	+	+
Fungimar	Cuprous oxide -do-	0.2	+	+
Phygon (dichlone)	2, 3-dichloro-1, 4 napthoquinone	0.2	+	+
Parzate (liquid) (Nabam)	Disodium ethylene bisdithiocarbamate (22 per cent)	0.2	—	—
		0.1	—	+
		0.05	—	
		0.025	+	
Flit 406 (captan)	n-(trichloromethylmercapto) -4-cyclo-hexene-1, 2-dicarboximide	0.2	+	+
Thiram	bis (dimethyl thiocarbamoyl) disulphide	0.2	+	+
Formaldehyde	Formalin	0.2	+	—
		0.1		+
Panogen	Cyano (methylmercuri) guanidine	0.2	+	—
		0.1		+
Rhizoctol	Methyl arsenic sulphide	0.2	+	—
		0.1		+
Tritisan	Pentachloro nitrobenzene	0.2	+	+
Vapam	Sodium methyl dithiocarbamate	0.2	—	—
		0.1	+	—
		0.05		+
Kerol	Not known	0.2	+	—
		0.1		—
		0.05		+
Control	—	—	+	+

N.B. (+) denotes growth of mycelium ; (—) denotes no growth.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the case of effective fungicides, the doses were reduced and the minimum dose which completely inhibited the growth of these fungi, was determined.

The doses of fungicides used and the data with regard to their efficacy against *Rhizoctonia bataticola* and *Sclerotium rolfsii* are presented in Table.

It is evident that among all the fungicides tested for the control of *Rhizoctonia bataticola*, only Parzate liquid and Vapam were effective at a dosage of 0.2 per cent. These fungicides were later tested at lower concentrations and it was found that while Parzate liquid was effective at 0.05 per cent, Vapam at this dose failed to check the growth of the fungus. Table shows that more fungicides can be chosen for the control of *Sclerotium rolfsii*. Parzate (liquid), Formaldehyde, Panogen, Rhizoctol, Vapam and Kerol were all effective at 0.2 per cent concentration. The minimnm effective dose for each of these fungicides was determined and is presented in the Table.

The interesting aspect of these investigations is that Parzate (liquid) and Vapam are both effective for the control of *Rhizoctonia bataticola* and *Sclerotium rolfsii* but at different doses. Parzate (liquid) has been able to check the growth of *Rhizoctonia bataticola* and *Sclerotium rolfsii* at a dosage of 0.05 and 0.2 per cent respectively while Vapam has shown its effectiveness against these fungi at 0.2 and 0.1 per cent concentration. Kerol which has proved effective against *Sclerotium rolfsii* at a dosage of 0.1 per cent, did not show any inhibitory effect against *Rhizoctonia bataticola* even at double the concentration. Out of the remaining fungicides, Formaldehyde, Panogen and Rhizoctol gave best results for the control of *Sclerotium rolfsii*. Abeygunawardena and Wood[1] while testing the efficacy of a few fungicides for the control of *Sclerotium rolfsii*, observed that Formalin when used as a soil drench gave 100 per cent kill of the sclerotia.

SUMMARY

The relative efficacy of fourteen fungicides was tested for the control of *Rhizoctonia bataticola* and *Sclerotium rolfsii* which are common soil-borne fungi. Parzate liquid (Disodium ethylene bisdithiocarbamate) and Vapam (Sodium methyl dithiocarbamate) were effective against both fungi while Formaldehyde (Formalin), Panogen (cyanomethylmercuri guanidine), Rhizoctol (Methyl arsenic sulphide) and Kerol gave best results against *Sclerotium rolfsii* only.

ACKNOWLEDGEMENTS : The authors are gratefull to Dr. R.S. Vasudeva and Dr. B.L. Chona for their keen interest during these investigations and for going through the manuscript. Thanks are also due to Dr. J. S. Grewal for helpful suggestions.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

[1]Abeygunawardena, D.V.W. and Wood R.K.S. *Phytopathology*, **47** : 607-609 (1957).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECT OF SPORE AGE AND CULTURE MEDIA ON THE DEGREE OF INFECTION BY USTILAGO AVENAE

AVDHESH NARAIN*

(Accepted for publication July 17, 1964)

INTRODUCTION : In the past, various races of oat smuts *Ustilago avenae* (Pers.) (Rostr.) and *U. kolleri* Wille have been hybridized on the culture media and their pathogenicity has been studied on oat varieties (Halisky, 1956; Holton, 1956, 1959; Holton and Halisky, 1960). The problem of loss in pathogenicity by spores produced on culture media was not reported by early workers. However, in the present studies results from an exploratory test indicated that the culture medium and/or spore age affected the degree of smut infection. Therefore, it seemed desirable to conduct experiments to determine the effect of culture media and spore age on the pathogenicity of F_2 progeny of an interracial hybrid. It has been observed that there is a variable degree of smut infection incited by hybrid and selfed spores. Therefore to evaluate the data it was considered necessary to use interracial hybrids as well as selfed spores (monochlamydospores) in the present study.

MATERIAL AND METHODS : Two experiments were conducted to test the effect of spore age and culture media on the degree of oat loose smut infection on 2 selected oat varieties. Atlantic (C.I. 4599) and Victorgrain (C.I. 7125) oat varieties were used to test the reaction of interracial hybrids and selfed spores of loose smut races A 14 and A 22. According to previous reports (Luke *et al.*, 1960, 1961) Atlantic is susceptible to race A 14 and Victorgrain is susceptible to A 22. The smut material of recently described (Luke *et al.*, 1961) races A 14 and A 22 of *U. avenae* was obtained from late Mr. S. J. Hadden of Pedigreed Seed Company, Hartsville, South Carolina, U.S.A. Seeds of Atlantic and Victorgrain oats were obtained from Aberdeen, Idaho, U.S.A.

In the initial experiment, hybrid spores were grown on potato dextrose agar, potato dextrose-Victorgrain extract agar and Victorgrain extract-water agar. The latter may be regarded as specialized media. It was found that potato dextrose-Victorgrain extract medium maintained higher pathogenicity of inoculum as compared to potato dextrose and Victorgrain extract-water agar. Consequently, the use of latter two media was discontinued and only potato dextrose-Victorgrain extract medium was used in the second experiment. Ingredients of potato dextrose-Victorgrain extract agar consist of : potato dextrose agar (Difco) 39 g., leaf extract from 40 g. Victorgrain oat leaves and 1 litre of distilled water. Victorgrain extract-water agar includes extract from 40 g. Victorgrain oat leaves, 25 g. Bacto Agar (Difco) and 1 litre of distilled water. Potato dextrose agar (Difco) dissolved in 1 litre of distilled water was used for non-specialized medium. For both specialized media

*Present Address : Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the Victorgrain oat leaves obtained from 4 week old seedlings were extracted with a measured quantity of hot distilled water in a waring blendor for 10 minutes. Leaf extract was filtered and mixed with other ingredients, making up the final volume to 1 litre. The media were sterilized by conventional methods in an autoclave.

For hybridizing races A 14 and A 22 of *U. avenae,* single chlamydospores of each pathogen were isolated, with the aid of Fonbrune pneumatic micromanipulator. These spores were allowed to germinate on agar drops of the three aforementioned media within moist chambers. Single isolated chlamydospores of each smut were allowed to grow for about 24 hours till they produced the complete set of 4 haploid sporidia. Immediately after this period the agar drop with 4 primary sporidia was transferred to the respective agar plates. In this manner monochlamydospore transfers of both loose smut races were made in separate agar plates on each of 3 kinds of media. These developed within 1 week. Each monosporidial colony was isolated singly in fresh agar plates and allowed to grow till it attained considerable size after multiplication by vegetative budding.

Complete sporidial sets from single chlamydospores of races A 14 and A 22 were maintained on 3 nutrient media. Crosses were made by transferring the haploid sporidia of races A 14 and A 22 with a sterilized platinum loop in all possible compatible combinations. Compatibility among sexually mating types was visible by development of white mycelial "suchafaden" as described by Bauch (1932). F_1 diploid spores developed in culture after a period of 2 weeks. Several F_1 interracial hybrid spores were transferred to agar plates and allowed to grow till they developed the F_2 population by self fertilization. Only F_2 hybrids were used for inoculation purpose in this study since avirulence is dominant in oat loose smut (Halisky, 1956).

Selfed spores of races A 14 and A 22 were produced by isolating single chlamydospores with the aid of micromanipulator. Diploid spores developed in 7-10 days after the mating of compatible haploid sporidia.

Effect of age of diploid F_2 spore (hybrid and selfed) on the degree of smut infection was determined. For this purpose 10, 20 and 30 day old spores were used as the inoculum. Both the experiments were conducted in a growth chamber maintained at temperatures between 72-74° F. In an earlier study by author (1962) it was determined that this temperature range is most suitable for heavy infection by oat smut. Allison's (1932) partial vacuum technique for seed inoculation was used. The non-dehulled seeds of each oat variety were inoculated and planted (10 seeds/pot) in 6-inch pots. Four replications were used for each treatment. After germination, during the seedling stage in each replicate only 6 plants were maintained after pulling out the accessory ones. This was done to keep a uniform population of 24 plants for each treatment by ruling out the possibility of non-germination, of some seeds. Prior to the planting, soil used in pots was treated with methyl bromide.

RESULTS : The results (Table 1) indicate that 20 day old hybrid (A 14×A 22) spores as well as the selfed spores of race A 14 produce higher smut infection on the Atlantic oat variety in comparison to the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

10 day old spores. This trend was recorded from all the spores obtained from potato dextrose agar, potato dextrose-Victorgrain extract agar and Victorgrain extract-water agar. However, both kind of spores, hybrid as well as selfed which were grown on potato dextrose-Victorgrain extract agar incited a higher degree of infection than those grown on potato dextrose and Victorgrain extract-water agar. The results also indicate that hybrid (A 14 × A 22) spores irrespective of 3 kinds of culture media on which they are produced are more infectious than the selfed spores of each race. This is evidenced by as high as 100 per cent infection of 20 day old and 83 per cent infection by 10 day old hybrid spores (Table 1).

TABLE 1 : Percentage of smut infection* on Atlantic incited by interracial hybrid and selfed spores of *U. avenae* grown on 3 different culture media for 10 and 20 days

Age of spores in days	Potato dextrose agar			Potato dextrose-victorgrain extract agar			Victorgrain extract-water agar		
		Selfed			*Selfed*			*Selfed*	
	A14 × A22	A14	A22	A14 × A22	A14	A22	A14 × A22	A14	A22
10	61	50	0	83	67	0	65	33	0
20	89	67	0	100	94	0	78	72	0

*Each percentage consists of 4 replications of 6 plants.

Results (Table 2) pertaining to the degree of infection on Victorgrain oat also reveal that 20 day old hybrid spores as well as selfed spores of A 22 are more infectious than the 10 day old spores of both categories. Again, same trend of high degree of pathogenicity by 20 day old selfed and hybrid spores was not affected by their production on any of the three nutrient media.

TABLE 2 : Percentage of smut infection* on Victorgrain incited by interracial hybrid and selfed spores of *U. avenae* grown on 3 different culture media for 10 and 20 days

Age of spores in days	Potato dextrose agar			Potato dextrose-victorgrain extract agar			Victorgrain extract-water agar		
		Selfed			*Selfed*			*Selfed*	
	A14 × A22	A14	A22	A14 × A22	A14	A22	A14 × A22	A14	A22
10	28	0	30	61	0	33	39	0	15
20	83	0	61	89	0	67	61	0	45

*Each percentage consists of 4 replications of 6 plants.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

From the second experiment, results (Table 3) indicate that 20 day old hybrid and selfed spores of each race produced highest percentage of smut infection. Comparatively, among both categories of spores, 10 day old were more infectious than the 30 day old ones.

TABLE 3 : Percentage of smut infection* on Atlantic and Victorgrain incited by interracial hybrid and selfed spores of *U. avenae* grown on potato dextrose-victorgrain extract agar for 10, 20 and 30 days

	Atlantic			Victorgrain		
		Selfed			*Selfed*	
Age of spores in days	A14×A22	A14	A22	A14×A22	A14	A22
10	84	80	0	73	0	53
20	93	87	0	85	0	62
30	55	50	0	26	0	20

*Each percentage consists of 4 replications of 6 plants.

As a result of smut infection the diseased plants of Atlantic oat variety were considerably stunted in comparison to the healthy plants. In addition, it was also observed that the diseased panicles in each variety developed about 7-10 days earlier than the healthy panicles.

DISCUSSION : The spores produced on potato dextrose-Victorgrain extract agar were more infectious than those produced on potato-dextrose agar or Victorgrain extract-water agar. Since the latter two media contain all ingredients of mixture, increased infection cannot be attributed to any single component (Victorgain extract or potato dextrose). Therefore, the increased pathogenicity of spores grown on potato dextrose-Victorgrain extract agar might be due to a balance of nutritional factors in this medium.

The 20 day old spores were more infectious than 10 or 30 day old spores. Apparently, the immaturity accounts for low infection caused by 10 day old spores whereas lack of viability owing to the staling products in culture media was responsible for the lowering of infection by 30 day old spores. Higher degree of oat loose smut infection incited by the hybrid spores may be explained as a result of the combination of genetic factors contributed by both races in the process of hybridization.

Results of this investigation reveal that for attaining high degree of oat loose smut infection, spore (hybrid and selfed) age and the culture media on which they are grown are of extreme importance. These characterstics are essential and facilitate the studies pertaining to the inheritance of pathogenicity in *U. avenae*.

SUMMARY

The effect of spore age and culture media on pathogenicity of races A 14 and A 22 of oat loose smut, *Ustilago avenae* was determined.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Progeny (F_2) from interracial hybrids and selfed (monochlamydospores) were grown on potato dextrose agar, potato dextrose-Victorgrain extract agar and Victorgrain extract-water agar for 10, 20 and 30 days. The interracial F1 hybrid (A 14 × A 22) spores grown on potato dextrose-Victorgrain extract agar incited the highest degree of smut infection on 2 susceptible oat varieties. Results indicated that the increased pathogenicity was due to balanced nutritional factors of potato dextrose-Victorgrain extract agar. Twenty day old spores were more infectious than 10 or 30 day old spores. Diseased plants were stunted in growth and matured earlier.

ACKNOWLEDGEMENT : The work reported herein is a portion of dissertation submitted by the author for Ph. D. degree in Plant Pathology at the University of Florida, U.S.A. Deep gratitude is expressed to Dr. H. H. Luke under whose direction this study was carried out. Appreciation is also extended to Dr. P. Decker, Head of Plant Pathology Department, University of Florida for providing the necessary facilities.

Department of Plant Pathology,
University of Florida,
Gainesville, Florida, U.S.A.

REFERENCES

ALLISON, C. C. (1937) Studies on the genetics of smuts of barley and oats in relation to pathogenicity. *Minnesota Agri. Exp. Sta. Tech. Bull.* **119** : 1-34.

BAUCH, R. (1932) Die Sexualitat von *Ustilago scorzonereae* und *Ustilago zeae. Phytopath. Z.*, **5** : 315-321.

HALISKY, P. M. (1956) Inheritance of pathogenicity in *U. avenae. Res. studies, State College of Washington*, **24** : 348-387.

HOLTON, C. S. (1956) Origin and production of morphologic and pathogenic strains of the oat smut fungi by mutation and hybridization. *J. agri. Res.*, **52** : 311.317.

HOLTON, C. S. (1959) Genetic controls of Host Parasite interactions in smut diseases. pp. 145-156. In Holton *et al.* (*Plant Pathology Problems and Progress* 1908-1958, Univ. Wisconsin Press, Madison.

LUKE, H. H., D. D. MOREY AND S. J. HADDEN (1960) Hosts for differentiating oat loose smut races of southeastern U.S. *Phytopathology*, **50** : 209-212.

LUKE, H. H., P. F. PFAHLER, AND S. J. HADDEN. (1961) Oat smut races of South Atlantic States. *Phytopathology*, **51** : 490-491.

NARAIN, A. (1962) *Physiologic studies of oat smuts.* 72 pp. Doctoral Dissertation, University of Florida, Gainesville, Florida, U.S.A.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFICACY OF DIFFERENT FUNGICIDES-VIII : FIELD TRIALS FOR THE CONTROL OF COVERED SMUT OF BARLEY (USTILAGO HORDEI (PERS.) LAGER.)

J. S. GREWAL AND DHARAM VIR

(Accepted for publication July 24, 1964)

Covered smut is an important disease of barley in India and occurs in parts of Bihar, Uttar Pradesh and Punjab causing considerable loss to the crop. The average loss in susceptible varieties varies from 2 to 5 per cent but in the years of epidemic it may be as high as 30 per cent. Mehta *et al.*,[4] estimated the losses in grain yield at about 2500 tons in Uttar Pradesh alone calculated on the basis of 2 per cent infection. Mehta[3] demonstrated the effectiveness of Agrosan GN and Ceresan for the control of this disease by seed treatment. But since then a large number of other proprietary fungicides based on a variety of active ingredients, have come into the market. It was, therefore, felt necessary that reliable information should be made available regarding the performance of some of the latest fungicides against covered smut of barley. An attempt has been made to test the relative efficacy of nineteen seed-dressing fungicides which included a variety of organo-mercurials, dithiocarbamates, quinones and heterocyclic nitrogen compounds for the control of this disease.

EXPERIMENTAL : Under natural conditions of disease development, it is not possible to get seed of uniform infection. Therefore, barley seed (variety N. P. 13) was artificially inoculated with viable spores of *Ustilago hordei* at the rate of 0.5 per cent of seed weight so that all the seeds have equal spore load and the relative efficacy of fungicides may be evaluated under similar conditions of infection. The seed was divided into twenty lots nineteen of which were treated with different fungicides at doses mentioned against each in Table 1 and the 20th was left as untreated control. An equal number of treated seeds were sown in plots of 8′×30′ in the 2nd week of November and the experiment was replicated four times. The field trials were carried out for a period of three years (1958-61) at the Agronomy Division farm. Observations were recorded with regard to the effect of fungicides on emergence, disease incidence and yield of crop and the data are summarised in Table 1.

The results in the Table indicate that the highest germination of 55.9, 54.6 and 58.2 per cent was obtained with ceresan for three consecutive years which represented an increase of 2.5-13.7 per cent over the untreated controls. The disease was completely controlled when seed was treated with ceresan or Agrosan 5W whereas the incidence of disease in the untreated control plots ranged from 14.3-17.9 per cent. Ceresan-treated seed gave an increased yield of 13.9-25.4 per cent against the untreated controls in different years. Machacek[2] compared the efficacy of some fungicides against seed-borne smut infection of some cereals at fifteen stations in Canada and seven in U.S.A. and found Ceresan to be

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Effect of seed-dressing fungicides on germination, disease incidence and yield of barley

Fungicide	Active ingredient	Dose % of seed weight	% Germination			% incidence of disease			Yield in kgs.		
			58-59	59-60	60-61	58-59	59-60	60-61	58-59	59-60	60-61
Arasan	bis (dimethylthiocarbamoyl disulphide)	0.3	55.6	53.4	58.0	0.8	2.0	1.2	8.3	8.9	10.0
N. I. Ceresan	ethylmercury phosphate	0.07	52.2	51.7	57.7	0.8	13.9	2.4	7.6	8.5	9.9
2% Ceresan	ethylmercury chloride	0.2	49.5	49.2	56.5	2.6	1.0	7.5	7.4	8.7	9.0
Cerenox	organic compound	0.22	48.2	48.4	55.1	1.4	9.3	10.5	7.1	8.5	9.4
Harvesan	organically combined mercury	0.3	53.3	47.3	55.4	1.6	9.1	6.8	7.7	8.0	8.8
Spergon	tetrachloro-p-benzoquinone	0.22	52.1	50.9	54.7	0.7	5.1	2.7	7.5	8.8	9.3
Tillex	ethylmercury chloride	0.2	49.4	53.5	54.9	1.0	5.5	2.3	7.2	9.0	9.4
Thiram	bis (dimethylthiocarbamoyl disulphide)	0.3	50.4	51.0	55.4	0.9	6.0	1.8	7.4	8.6	9.5
Lunasan	1-(ethylmercury)-2-thiourea	0.3	55.0	52.3	56.6	6.0	9.5	6.8	8.1	8.9	8.9
Phygon	2-3 dichloro-1, 4-napthoquinone	0.15	49.8	52.2	56.0	1.6	4.0	3.9	7.4	8.9	9.0
Fusariol	ethylmercury cyanide	0.2	52.9	51.0	55.3	0.4	0.3	0.8	7.9	8.8	9.7
Ceresan	phenylmercury acetate	0.25	55.9	54.6	58.2	nil	nil	nil	8.4	9.0	10.0
Agrosan GN	tolylmercury acetate	0.3	50.8	49.1	57.4	0.4	6.7	1.4	7.3	8.9	10.0
Fernasan A	bis (dimethylthiocarbamoyl disulphide)	0.3	48.9	52.0	55.9	1.2	1.2	2.7	7.2	8.7	9.1
Flit 406	n-(trichloromethylmercapto)-4-cyclohexene-1, 2-dicarboximide.	0.2	53.2	49.7	56.8	0.5	1.7	1.9	7.7	9.0	9.6
Hexasan	organically combined mercury	0.3	51.9	54.0	55.7	0.6	3.0	3.0	7.3	9.0	9.0
Leytosol B	phenylmercury urea	0.07	51.9	49.7	54.8	0.7	16 8	1.4	7.7	8.8	8.5
Tritisan	pentachloronitrobenzene	0.07	52.9	51.5	54.2	0.4	16.7	0.7	7.5	8.2	9.1
Agrosan 5W	Organically combined mercury	0.07	51.8	49.0	53.2	nil	nil	nil	7.3	8.6	9.0
Control	— —	--	42.2	52.1	52.7	14.3	17.9	17.2	6.7	7.9	8.5

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

effective against covered smut of barley. Similar results were obtained by Leukel[1] with Ceresan in field fungicidal trials in southern states of U.S.A.

Although Agrosan 5W has given complete control of disease, the results regarding its effect on germination are not very encouraging. This fungicide is also not yet available in this country. Therefore, till some new and better fungicides come into the market, Ceresan can be used for effective control of the disease. The cost of seed treatment with this fungicide is about 50 nP. per acre.

SUMMARY

Nineteen seed-dressing fungicides *i.e.*, Arasan, N. I. Ceresan, 2 % Ceresan, Cerenox, Harvesan, Spergon, Tillex, Thiram, Lunasan, Phygon, Fusariol, Ceresan, Agrosan GN, Fernasan-A, Flit 406, Hexasan, Leytosol B, Tritisan and Agrosan 5W were tested in the field for the control of covered smut of barley (*Ustilago hordei*) over a period of three years under artificial conditions of seed infection. Ceresan and Agrosan 5W gave complete control of disease. Highest germination was, however, recorded with Ceresan which was 2.5-13.7 per cent more than the untreated control.

ACKNOWLEDGEMENT : We are deeply grateful to the Head of the Division of Mycology and Plant Pathology for keen interest and helpful suggestions. Thanks are also due to Shri Raghubir Singh, Junior Scientific Assistant for assistance in the field operations.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

[1]Leukel, R. W. *Plant Dis. Reptr.*, **33** : 295-299, 1949.

[2]Machacek, J. E. *Plant Dis. Reptr.*, **35** : 146-148, 1951.

[3]Mehta, P. R. *Agric. Anim. Husb. Uttar Pradesh*, **2** : 3-7, 1951.

[4]Mehta, P. R., B. Singh, S. C. Mathur and S. B. Singh *Sci. and Cult.*. **19** : 262-63, 1953.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SORODISCUS RADICICOLUS COOK, A NEW RECOD FOR INDIA

M. S. GHEMAWAT

(Accepted for publication May 25, 1964)

In August, 1959, lateral roots of *Gynandropsis gynandra* (Linn.) Brig.=*G. pentaphylla* DC., collected in the Botanical Garden at the University of Jodhpur, Jodhpur, were found to be parasitized by an organism which caused conspicuous malformations or galls (fig. 1). An examination of these galls from material dissected in lactophenol as well as from hand-cut and microtome sections (10 μ) stained with saffranin and fast green revealed that they are caused by a plasmodiophorous fungus which appears to be indentical to *Sorodiscus radicicolus* Cook. Cook (1931)* reported this species as causing tumours on the roots of *Gynandropsis pentaphylla* in South Africa and described it was a new species of the Plasmodiophoraceae. The Indian fungus differs in some minor characteristics from Cook's species. The present fungus is regarded for the time being as identical with *S. radicicolus*, but a more detailed study of the life history, cytology and host range of the fungus, is being made to determine whether or not these differences are taxonomically significant. The results of this study will be reported in a snbsequent publication.

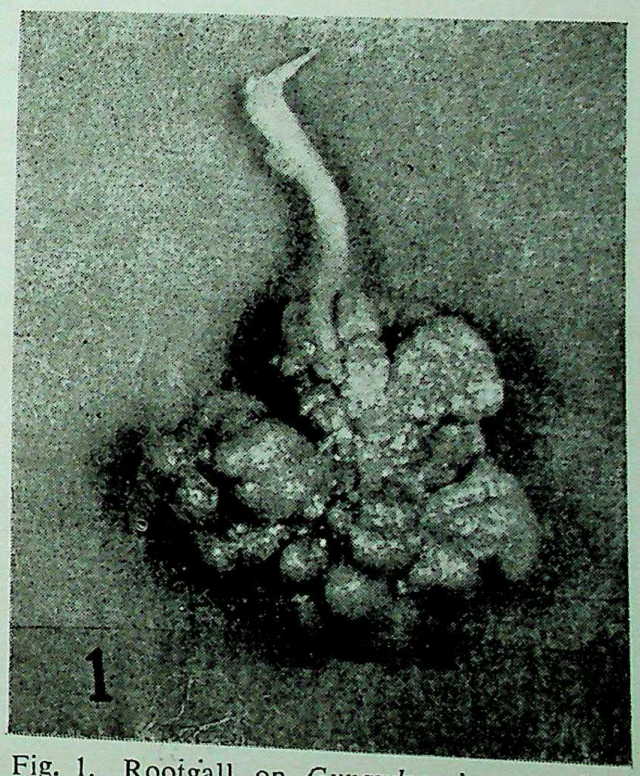


Fig. 1. Rootgall on *Gynandropsis gynandra* caused by *Sorodisocus radicicolus* ×2.

*Cook, W. R. Ivlmey, *Ann. Mycol. Berl.*, **29** : 313-324, (1931).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The galls caused by this fungus on the lateral roots are whitish to pale yellow, convoluted and coral-like in shape and vary from 2 to 23 mm. in diameter (fig. 2). As they mature and become old, they may turn reddish brown in colour. The central cylinder of the roots is not attacked by the fungus and the galls, which originate in the cortex, are formed primarily as the result of cell division.

Fig. 2. Rootgalls of *S. radicicolus* of different sizes, × 1.

The intracellular fungus is confined to the parenchymatous cortical cells where it develops cystosori during the cystogenous phase. These cystosori are circular to ovoid or slightly irregular in outline and appear as flattened discs which are composed of two compact lyers of cysts or resting spores (figs. 4 and 5). In sections of mature galls, almost all the cystosori are flattened and their two-layered nature is not always discernible. The mature cystosori vary from 13—34 × 13—29μ in diameter, with a mean of 22.5 × 17μ, and from 7 to 9μ in thickness. The number of cysts or resting spores per cystosorus varies from 35 to 65, and these appear ovoid, almost rectangular or polygonal in outline in median sections. They range in size from 3.3—4.7 × 3.3—4.4μ, with a mean of 4 × 3.8μ. Their walls are thick and smooth, and their free or external portions may extend outwards to from attenuated pegs or spines, similar to those figured by Cook.

As noted above, minor differences from Cook's fungus were found in the Indian species. Cook reported the galls to vary from 3—15 mm. in diameter, whereas in Indian material they ranged from 2 to 23 mm. Also, he found that the cystosori were upto 25 × 12μ in diameter and contained upto 50 resting spores which measured 3.8—4.2 × 3.2—3.6μ. In the Indian material, the cystosori varied from 13—34 × 13—29μ in diameter and contained 35 to 65 resting spores which ranged from 3.3—4.7 × 3.3—4.4μ. Cook also reported that the young cystosori are enveloped by a soral membrane and that the cysts separated readily even before the disintegration of the galls. No enveloping soral membrane was present in the Indian material (fig. 3) and it is not improbable that Cook might have mistaken the surrounding host cytoplasm for a membrane. Furthermore, it was found that even at maturity and after the galls had disintegrated, the resting spores did not separate in the Indian fungus. These differences may not prove to be significant from the taxonomic standpoint.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

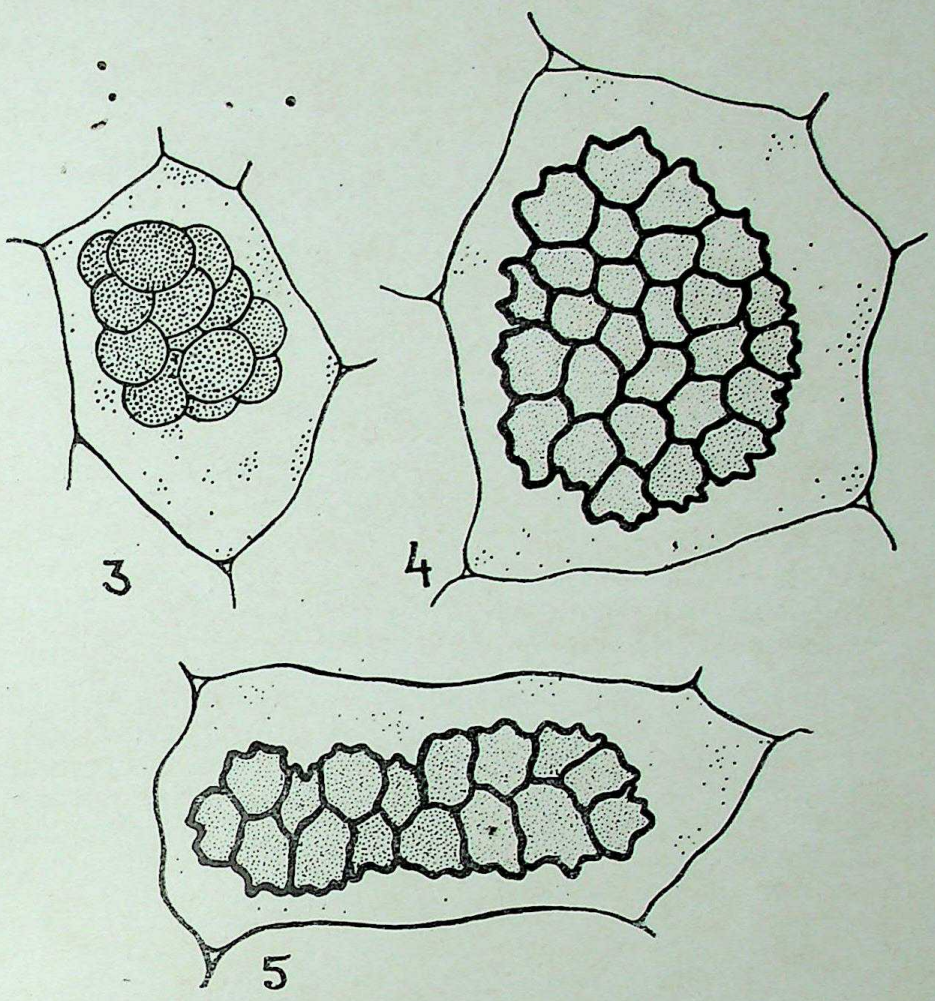


Fig. 3. Young cystosorus of *S. radicicolus* in the host cell. × 1500.
Fig. 4. Section of a mature cystosorus of in the host cell, in flattened view. × 1500.
Fig. 5. Section of a mature cystosorus in the host cell, in side view. × 1500.

This is the first record of the genus *Sorodiscus* Lagerh. and Winge from India.

Summary

Sorodiscus radicicolus Cook is reported for the first time from India on *Gyanandropsis gynandra*. It shows certain minor differences from the original description of the fungus from South Africa.

Acknowledgements : The author expresses his grateful thanks to Prof. J. S. Karling, of Purdue University, Lafayette, U.S.A., for confirming some of the views of the author about the fungus and for going through the manuscript. Thanks are also due to Dr. H. C. Arya, University of Rajasthan, Jaipur and Dr. B. Tiagi, University of Jodhpur, Jodhpur.

Department of Botany.
Government College,
Kota (Rajasthan).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PATHOGENICITY AND VARIETAL REACTION OF PADDY TO SCLEROTIUM ORYZAE CATT. IN BIHAR

A. P. MISRA AND ABU MOHAMMAD

(Accepted for publication July 26, 1964)

Stem rot of paddy, *Sclerotium oryzae* Catt. has been reported practically from every rice-growing country of the world. Shaw made the first Indian collection of the disease in Noakhali (now in Pakistan) in 1911. Since then the disease has been reported from Madras (Shaw, 1913), Punjab (Luthra and Sattar, 1936; Paracer and Luthra, 1944), Assam (Nandi, 1941), Madhya Pradesh (Nikam, 1954) and Bihar (Shaw, 1913; Butler, 1918).

Mundkur (1935) found *S. oryzae* to be pathogenic on rice seedlings, but under field conditions it was considered to be a weak parasite. Luthra and Sattar (1936) and Paracer and Luthra (1944) however, found it to be pathogenic under field conditions.

During 1962 and 1963, the fungus was widespread on paddy in the months of October-November in the Shahabad district of Bihar, especially in the areas under Intensive Agricultural Development Programme at Bikramgunj and Buxar, where the variety 2206-B (BR 34) was mostly grown. The sclerotia of the fungus were found in abundance inside the culms and the basal leaf sheaths, and a grey weft of mycelium was seen on splitting open the diseased culm.

Investigations were taken up to test the pathogenicity of the fungus and study the reactions of the paddy varieties commonly grown in Bihar at different stages of plant growth.

MATERIAL AND METHODS : The fungus was isolated from diseased culms and brought into pure culture by planting a single sclerotium on potato-dexrose-agar. The inoculum was multiplied on sterilized soil-oats or soil-maize medium (soil 95g, crushed oats or maize 5g.).

Soil Inoculation : The inoculum was mixed in the upper layer of steam-sterilized soil, seven days before sowing the seed or transplanting of seedlings. In varietal tests at tillering stage, the inoculum was placed in the soil round the base of the stem after scraping the soil.

Pathogenicity tests (*Seedling stage*) : These were carried out in (i) 1″ diameter hard glass test tubes and (ii) 250 ml. beakers. Water-logging conditions, as found in fields, were maintained.

The test tubes and beakers were half-filled with sun-dried soil and covered with muslin cloth. These were then sterilized and inoculated with *S. oryzae*. Paddy seeds of variety 2206-B, surface disinfected with 0.1 per cent mercuric chloride solution, washed in 3 to 4 changes of sterile water, were sown in test tubes and beakers seven days after inoculation. In another set, seeds were sown in uninoculated soil which served as control.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Varietal reaction : Reaction of paddy varieties 115 BK, 141 BK, 16 BK, 88 BK, 36 BK, 498-2A, 818-3, 2206-B, CH 10 and CH 1007, was studied in pots at three stages.

1. *Seedling stage* : 25 seeds were sown in 4″ pots in inoculated soil (16 g. inoculum applied to 100 g. of sterilised soil). Data on the percentage of stem rot were recorded after 21 days.

2. *After transplantation* : Four (3-week old) seedlings were transplanted in 4″ pots in inoculated soil as above. Readings were taken after 30 days when the plants began to dry up and rotting of culms was observed near the soil surface.

3. *Tillering stage* : Two (3-week old) seedlings were transplanted in 9″ pots; inoculum was applied two weeks after transplantation at the same rate. Observations were taken after eight weeks. Cement was applied at the bottom and the outer surface of pots to maintain water-logging conditions.

Experiments 1 and 2 were repeated using unsterilized soil from pots which had earlier been inoculated.

RESULTS : *Pathogenicity tests* : The seedlings emerging from inoculated soil dried up in 2 weeks' time. Sclerotia were formed on rotten culms. On the other hand the control plants did not show any symptoms and were growing vigorously (Fig. 1).

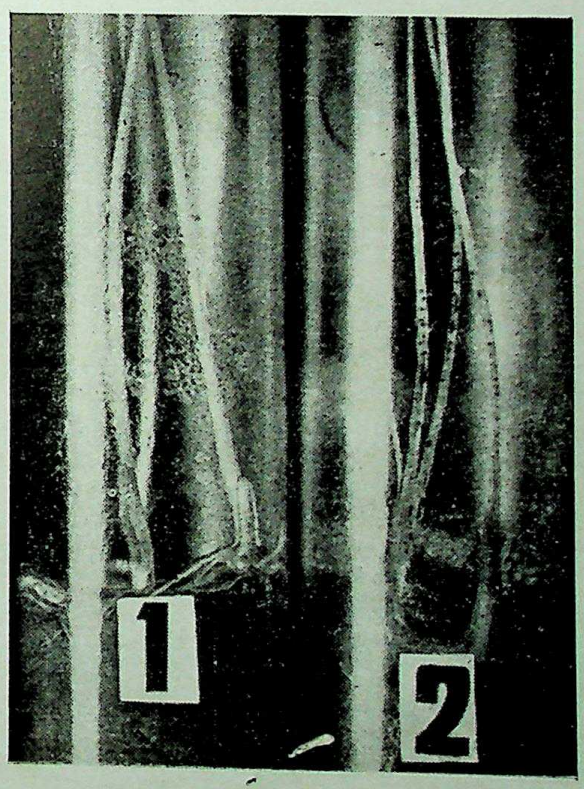


Fig. 1. Pathogenicity of *S. oryzae* (1) Uninoculated ; (2) Inoculated (Showing sclerotia) ;

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Varietal reaction : 1. *Seedling stage* : None of the varieties proved resistant, though there were differences in susceptibility. In unsterilized soil more or less similar results were obtained.

2. *After transplantation* : The varieties 142 BK, 88 BK, 36 BK, developed stem rot symptoms with sclerotia inside the culms in all the 12 plants, but in varieties 498-2A, 16 BK, and 115 BK, 4, 5, and 6 plants respectively were infected. In the remaining varieties 9 to 11 plants were affected. None of the varieties proved immune. More or less similar results were obtained in unsterilized soil.

TABLE 1 : Infection* due to *Sclerotium oryzae* in different paddy varieties at different stages of plant growth

Varieties	Seedling stage	After transplantation	Tillering stage
	Percentage infection	No. of plants infected** out of 12	No. of plants infected** out of 6
115 BK	58.28	6	6
141 BK	90.00	12	6
16 BK	56.56	5	3
88 BK	61.26	12	4
36 BK	66.79	12	3
498-2A	73.80	4	0
818-3	78.95	10	5
2206-B	62.91	11	0
CH 10	62.66	10	0
CH 1007	52.80	9	0
Uninoculated control	0	0	0

*Average of three replicates.
**Percentages of infections were not calculated as the plants were few in number.

3. *Tillering stage* : Stem rot developed in varieties 115 BK, 141 BK, 16 BK, 88 BK, 36 BK and 818-3. Sclerotia were found on splitting the culm. The varieties 498-2A 2206³-BCH 10 and CH 1007 did not develop any infection. The minimum and maximum temperatures during the period of experiment ranged from 24.7°C to 33.8°C and the relative humidity from 71 per cent to 96 per cent.

DISCUSSION AND CONCLUSION : The pathogenicity of *Sclerotium oryzae* to paddy was established both in the seedling and transplanted stage in laboratory and greenhouse under water-logged conditions. Shaw (1913), Butler (1918) and Mundkur (1935) obtained heavy infection on rice seedlings grown in test tubes, but under natural conditions the fungus was at the most to be regarded as a weak parasite. On the other hand Luthra and Sattar (1936) and Paracer and Luthra (1944) showed the pathogenicity of *S. oryzae* and carried out tests for varietal

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

reactions under natural conditions. The difference in the results in the present set of experiments from those of Mundkur at Pusa in 1932, 1933 and 1934 may either be due to the water-logged conditions maintained in pot trials or to a different and more pathogenic strain of the fungus studied.

Varietal reaction at three stages in sterilized and infected soil was studied. Out of ten varieties 115 BK, 141 BK, 16 BK, 88 BK, 36 BK, 498-2A, 818-3, 2206-B, CH 10 and CH 1007 tested for infection, at seedling and after transplantation, none of the varieties proved resistant, though there were differences in the percentages of infection.

Among the ten varieties tested for infection at tillering stage. Varieties 115 BK, 141 BK, 16 BK, 88 BK, 36 BK and 818-3 developed stem rot symptoms with sclerotia inside the culms.

ACKNOWLEDGEMENT : Thanks are due to Sri R. S. Roy, Principal, Bihar Agricultural College, Sabour for granting facilities for this work.

Department of Mycology and Plant Pathology,
Bihar Agricultural College,
Sabour (Bhagalpur).

REFERENCES

BUTLER, E. J. (1918) *Fungi and disease in Plants*, Thacker, Spink and Co., Calcutta.

LUTHRA, J. C. AND A. SATTAR, (1936) Some studies on the sclerotial disease of rice (*Sclerotium oryzae* Catt.) in the Punjab. *Indian J. agric. Sci.*, **6** : 973-984.

MUNDKUR, B. B. (1935) Parasitism of *Sclerotium oryzae* Catt. *Indian J. agric. Sci.*, **5** : 393-414.

NANDI, H. K. (1941) Annual Report of the Economic Botanist Assam for the year 1939-40 (*Rev. Appl. Mycol.* **20** : 447).

NIKAM, B. G. (1954) Diseases of paddy in Madhya Bharat in 1953-54. *Plant Prot. Bull.*, **6** : 33.

PARACER, C. S. AND J. C. LUTHRA, (1944) Further studies on stem rot disease of rice caused by *S. oryzae* in the Punjab. *Indian J. agric. Sci.*, **15** : 44-48.

SHAW, F. J. F. (1913) A sclerotial disease of rice. *Mem. Dep. Agri. India*, Bot. Ser., **6** : 11-23.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SEED ROT OF HEVEA BRASILIENSIS CAUSED BY BOTRYODIPLODIA THEOBROMAE PAT.

S. N. S. SRIVASTAVA

(Accepted for publication July 29, 1964)

Massee (1910) remarked that *Diplodia rapax* Mass., which is perhaps same as *Botryodiplodia theobromae* Pat. (Petch, 1910), might have been carried on *Hevea* seed from one country to another. Petch (1910, 1911) stated that *B. theobromae* occurred on *Hevea* pods in Ceylon as a saprophyte. La Rue and Bartlett (1923) recorded that the same fungus was often responsible for considerable deterioration of the seeds in Sumatra (Indonesia), and described some of its cultural characters and results of pathogenicity tests on certain hosts. Gorenz (1953) reported the fungus on maturing seed pods in Mexico. Recently, Srivastava (1956) stated that about 10 per cent of *Hevea* seeds, imported from Malaya, were often found to be affected by a rot due to the same fungus.

B. theobromae also occurs on *Hevea* seeds in India. An examination by the author of 4 lots of 100 seeds each, from 4 different plantations in southern India, showed about the same incidence of the fungus as on the seeds from Malaya. Some studies on the morphology, pathogenicity (on *Hevea* seeds) and growth rate (on artificial media) of two isolates of the fungus from Indian seeds indicated a resemblance between them and the Malayan isolates. Results of work undertaken so far, on the fungus as well as the disease it incites, are reported in the present paper. The work was conducted with seeds of Malayan origin as they were conveniently available.

Symptoms of disease : The affected seeds hardly show any symptoms on the outside, except in a few cases when they may be lighter and with a pinkish dorsal surface. The various stages of infection become apparent on splitting open the affected seeds (Fig. 1). In the earliest stage (Fig. b), there is a slight browning of the endosperm as compared to that of the healthy seed (healthy, Fig. 1a). Later, both the embryo and the endosperm are turn brownish to black, the change in colour usually commencing from the hilum end of the seed (Fig. 1c, d). In advanced stages (Fig. 1e, f), a blackish felt-like layer, about 1 mm. thick and largely composed of fungal hyphae, is also noticed surrounding the endosperm, underneath the testa. Severely diseased seeds show various stages of disintegration of the embryo and the endosperm (Fig. 1 g, h, i).

Isolation and identity of the disease organism : Pure cultures were obtained on potato-dextrose agar by plating out portions of tissue from affected seeds, after surface-sterilisation with 1/1000 mercuric chloride solution in water for 40-45 seconds. Hard, stromatic masses of pycnidia appeared in cultures of fresh isolates in about 7-10 days. Within 3 weeks, more or less mature pycnidia and spores, closely resembling those of *B. theobromae* Pat. (Patouillard and Lagerheim, 1892 ; Petch, 1911), were found to be embedded in the mycelial felt in culture.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

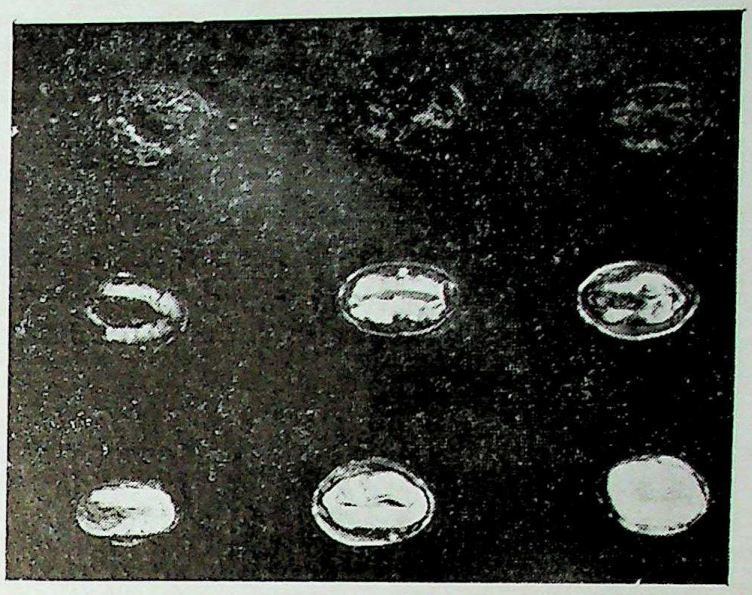


Fig. 1. Symptoms of *Botryodiplodia theobromae* rot of *Hevea* seeds in nature. Rows froms left to right : Top : a, b, c ; Middle : d, e, f ; Bottom g, h, i. (For explanation see text).

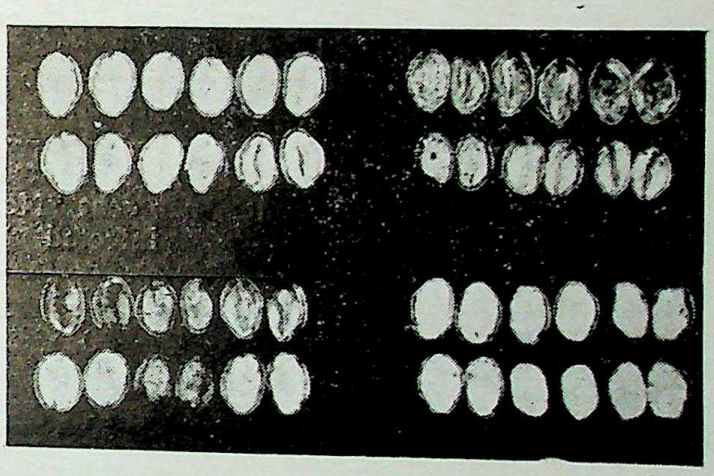


Fig. 2. Symptoms of *B. theobromae* rot of *Hevea* seeds on artificial inoculation. Top (left) : Control—uninoculated. (right) : Inoculated with spore suspension. Bottom (left) : Inoculated with mycelium. (right) : Soaked in spore suspension (unwounded).

Pathogenicity of the isolate : Surface-sterilised seeds were inoculated with the disease organism by immersing them in a spore suspension for 20 hours. Some of the seeds were also wounded before inoculation. Surface-sterilisation was carried out by dipping the seeds in rectified spirit for 1-2 seconds, followed by immersion in 1/1000 aqueous mercuric chloride solution for 30-40 seconds.

About 2-week-old seedlings growing in 6″ pots were also inoculated; some of them were injured prior to inoculation. Suitable controls were maintained.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results showed that the organism affected only the wounded seeds and seedlings. (Infection was noticed in 4 cases out of 6 in two tests, while in the third all the 6 injured seeds were diseased). The finding that the organism is a wound parasite is in accord with several of the earlier reports (Howard, 1901; Van Hall and Drost, 1909; Petch, 1910; La Rue and Bartlett. 1923).

Viability of the fungus : Viability tests showed that the disease organism remained alive even after 12 months as mycelium. Spores from pycnidia formed on seeds and also those on cultures lost their viability after 4 months.

Viability of seeds in relation to storage and infection of B. theobromae : Various lots of visually disease-free seeds were stored in sterile, moist saw-dust—which is often employed as a packing material for storage and transit of rubber seeds — for 7-21 days. In two cases, seeds showing pycnidia of the fungus on the surface, but otherwise apparently healthy, were also included. At the end of the desired storage period, known numbers were sown in fresh garden soil and their germination observed. The seeds which did not germinate were examined for the presence of infection of *B. theobromae*. The results are given in Table 1.

TABLE 1 : Percentage germinated seeds of *Hevea* after storage for varying periods and ungerminated seeds showing *Botryodiplodia* infection

Period of storage (days)	Number of seeds sown	Germinated per cent	Ungerminated per cent *Not infected	**Infected
0	12	33.5	58.4	8.3
0	10	70	30	0
7	10	50	50	0
8	10	50	50	0
11	10	30	70	0
17	10	50	30	20
20	***25	56	44	0
21	****40	72.5	22.5	5
Average 10.5	15.5	51.5	38.1	10.4

*Not infected with *B. theobromae*..

**Infected with *B. theobromae*.

***Of these seeds, 3 showed pycnidia on the surface; one of them germinated normally, but the other two did not germinate, nor did they exhibit any *Botryodiplodia* infection internally.

****9 of the seeds bore superficial pycnidia. Three of them produced normal plants; the rest did not germinate nor they exhibited any *Botryodiplodia* rot. The spores from pycnidia on some of these seeds were found to be viable even at the close of the experiment, after 3 months.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Only about 30-70 per cent (average 51.5 per cent) of the seeds usually germinated irrespective of whether they were sown soon after arrival in India, or after storage for periods up to 3 weeks. On an average, the failure of only about 10 per cent of the seeds to germinate was found to be due to *B. theobromae*.

It can also be seen from the Table that the presence of pycnidia of *B. theobromae* on the surface of the seeds is not necessarily associated with an internal rot, nor is merely the superficial infection responsible for a loss in their viability.

In view of these results, it appears probable that the fungus, which is known to attack the pods, passes on to the seeds through hilum, or otherwise, during the course of their development.

Control : The superficial infection of the fungus on the seeds has already been reported to be amenable to treatment with formaldehyde (Srivastava, 1956). To eliminate internal infection, heat therapy was tried :

1. Steam, at atmospheric pressure, passed over seeds for 2, 5 and 7 minutes.
2. Dry, hot air, at about 100°C., passed over the seeds for 5, 10 and 15 minutes.
3. Hot water, at 45°, 48° and 49°C., immersion for 15, 30, 45 and 60 minutes each.

Two dozens of each of apparently diseased and healthy seeds were treated alongside. The diseased seeds were surface-sterilized, halved, and plated out on potato-dextrose agar, to see the effect of the treatment on any infections that might be present inside the seeds. The healthy seeds were air-cooled and sown in ordinary garden soil in pots to study the effect of the treatments on the viability of the seeds. Suitable controls were maintained.

All treatments with steam and hot air resulted in complete loss of germination capacity of the seeds. The treatments with steam were effective in killing the internal infections too, but the hot air was effective only when it was passed over the seeds for 15 minutes.

TABLE 2 : Comparative germination of healthy seeds treated with hot water at various temperatures

Temperature (°C)	Time (minutes)	Per cent germination	Per cent control
a. No treatment (control)	—	50	0
b. 45	15	75	50
	30	25	50
	45	75	75
	60	50	25
c. 48	15	50	0
	30	50	0
	45	30	25
	60	40	70
d. 49	15	75	100
	30	75	100
	45	50	100
	60	50	100

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results of hot water treatment are given in Table 2. The 'percentage control' denotes the percentage of diseased seeds which did not yield any fungus on isolation.

Though the treatments at 45° and 48°C. were inconsistent the most promising treatment appeared to be immersion of the seeds in water at 49°C. for 15 minutes. But the results are not consistent here again as when it was further tried on two lots of 50 seeds each on two different occasions, germination of seeds fell down drastically (36 and 12.5 per cent in the two cases tried). Hot water treatment, therefore, cannot be adopted for eradication of seed infection. The only way open is to prevent pod and seed infection in the plantations themselves.

Summary

A seed rot of *Hevea brasiliensis*, caused by *Botryodiplodia theobromae*, is described. The spores *in situ* remain viable for about 4 months, while the mycelium remains so for 12 months.

On an average, only about 50 per cent of the imported seeds are viable, irrespective of whether they are sown immediately on arrival in India, or after storage in moist saw-dust for periods up to three weeks. The loss in the viability of about 10 per cent of the seeds has been found to be due to *B. theobromae* infection which seems to take place when the fruits are maturing on the tree.

Treatment of the seeds with hot water at 49°C. for 15 minutes, though promising, is erratic in its effect and drastically brings down the germination of seeds. It is suggested that the seed infection, which originates on the tree, be prevented by adopting suitable sanitary practices.

Acknowledgement : The writer is grateful to Dr. P. R. Mehta, Deputy Director (Plant Diseases), Directorate of Plant Protection, Quarantine and Storage, New Delhi, for suggestions and help in preparation of the manuscript, and to Dr. T. S. Sadasivan, Director, Madras University Botany Laboratory, for laboratory facilities. Thanks are also due to M/S. Harrisons and Crossfield Ltd., Quilon, for their co-operation in the work.

Directorate of Plant Protection,
Quarantine and Storage,
Plant Quarantine and Fumigation Station,
Madras 1.

References

*Anonymous (1950) Annual Report, West African Cacao Research Institute, April, 1948, to March, 1949, 64 pp.

Howard, A. (1901) *Diplodia cacaoicola* P. Henn. *Ann. Bot., Lond.*, **15** : 683-701.

La Rue, C. D. and H. H. Bartlett, (192?) *Diplodia* disease of *Hevea brasiliensis*. *Papers Michigan Acad. Sci., Arts and Letters*, **2** : 91-107.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

*GORENZ, A. H. (1953) *Diplodia* infection of *Hevea* buddings and its prevention by the use of a preventive fungicide. *Circ. U.S. Dep. Agric.* 913, 22 pp.

MASSEE, G. (1910) Fungi Exotici—X. *Kew Bull.*, **1** : 3.

*PATOUILLARD, N. AND C. V. LAGERHEIM, (1892) Champignons de l' Equateur. *Bull. Soc. Myc. France*, **8** : 136.

PETCH, T. (1910) On *Lasiodiplodia*. *Ann. Roy. bot. Gard., Peradeniya*, **4** : 445-465.

————, (1911) *The Physiology and Disease of Hevea brasiliensis*, 268 pp., DuLau and Co., Ltd., Londo.

SHARPLES, A. (1936) *Diseases and Pests of the Rubber Tree*. xv+480 pp. Macmillan and Co., London.

SRIVASTAVA, S. N. S. (1956) Two fungal infections of *Hevea* seeds. *Curr. Sci.*, **25** : 157-158.

*VAN HALL, A. E. AND A. W. DROST, (1909) De instervingsziekte der cacaoboomen en het "bruin rot" der cacaovruchten, veroorzaakt door *Diplodia cacaoicola*. *Bull. No. 21, Dep. van den Landbouw Suriname*.

*WARD, F. S. (1926) Inoculation experiments in relation to sun-scorch on exposed lateral roots of *Hevea brasiliensis*. *Malayan Agric. Jour.*, **14** : 286-289.

*YOUNG, H. E. (1954) Diplodia dieback and collar rot of *Hevea* and blue spot of crepe rubber. *Adv. Circ. Rubb. Res. Inst. Ceylon*, **44**, 4 pp.

*Original not seen.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

A New Stilbospora species from India—M. SHUKLA, BOTANY DEPARTMENT, D.A.V. COLLEGE, KANPUR. Leaves of a grass species bearing blackish, dot-like, spherical to enlongate spots on the stems and leaf sheaths were collected during a visit to Tanda Falls, Mirzapur, U. P. The material on examination revealed the presence of subepidermal pycnidia-like acervuili which contained characteristic phaeophragmoid, cylindrical or curved spores with truncate ends. The fungus seems to be near to *Coryneum* Nees ex Fries and *Steganosporium* Corda of Melanconiaceae and comes very close to *Stilbospora* Pers., which also is included in the same family. It differs from *Coryneum* in mass extrusion of spores from the pycnidia and from *Staganosporium* in colour and morphological details of the spores. About 27 species of *Stilbospora* are known. Two species have been reported from India (Ramakrishnan and Subramanian[2]). The present one differs markedly from all the described species. Whether the differences warrant a generic distinction or merely a specific one is to be decided. The author agrees to the suggestion of Sutton personal communication), and proposes to describe the fungus in question as a new species of *Stilbospora* rather than to add yet another-generic name to a group which already has for too many, some of them imperfectly delimited from others.

Stilbospora forms subepidermal, pycnidia-like acervuli which are black, carbonous, shining, spherical to elongate, scattered or aggregated. The olivaceous pluriseptate, cylindrical or curved spores with truncate ends, get liberated in mass when the fructification is slightly pressed in water. The pycnidium has a wall made up of several layers of which the outer are black and sterile and the inner hyaline and fertile bearing short conidiophores with conidia.

The present fungus differs from the other described species of *Stilbospora* in several respects. The conidia are very characteristic. The partitions dividing the conidial cell should better be called as false or pseudosepta. The cell lumen is reduced to various degrees, giving the appearance of partition brought about by true septa. Occasionally longitudinal septa are also present. Conidia have mostly truncate ends but it is not a fixed character for all the spores. The number of septa varies from 3-9. Conidiophores are short and seem to be annellate *i.e.*, producing conidia from a succession of apical growing point (Hughes[1]). Paraphyses are present. These are separate and comparatively longer with pointed or cylindrical ends. It is, therefore, proposed to create a new species with the name *Stilbospora graminicola* since it is found on a species of grass.

Stilbospora graminicola Shukla sp. nov.

Pycnidia erumpentia, linearia vel sphaerica, globosa vel conica, carbonacea, nigra nitentia, solitaria dispersa aggregata, ostiolata, unilocularia; parietes pseudoparenchymatei, consantes epluribus seriebus, quarum exterior fusce viridescens vel nigra, sterilis, interior velo hyalina, fertilis, supportans sporophora et paraphyses; pycnidia 0.25-1.0 mm.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Conidiophora magnitudinis variae, vulgo breviora, 6-25 μ longa, eracta, hyalina et annellata. Conidia fisce olivacea, 3-9 pseudoseptate, nonconstricta ad septa, cylindraces, curvata convexa-concave, apicibus obtusis, truncatis, magnit. 21-52 × 9-16μ. Sporae liberatae per saturam.

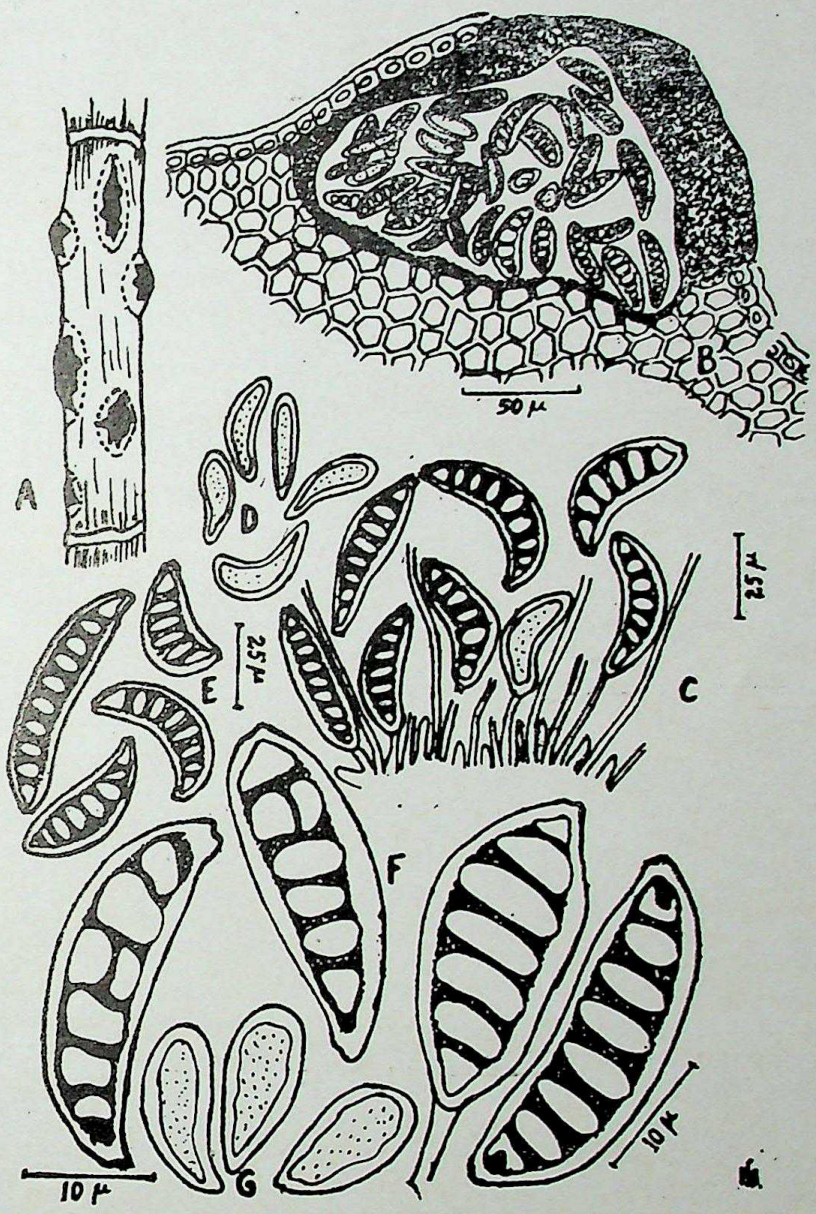


Fig. A. Pycnidia on stem (diagrammatic) Approx. ×15.
Fig. B. Pycnidium in vertical section.
Fig. C. Portion of the fertile layer showing conidia, conidiophores and paraphyses.
Fig. E and F. Conidia (Mature)
Fig. D. and G. Conidia (Young)

Pycnidia erumpent, linear, or spherical, globose to conical, carbonous, black, shining, solitary scattered or aggregated, ostiolate, unilocular, pycnidial wall pseudoparenchymatous, made up of several layers.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Outer dark greenish to black, sterile; inner hyaline, fertile bearing sporophores and paraphyses. Pycnidia measurig from 0.25-1.0 mm. Conidiophores variable in size, usually shorter, 6-25μ long, erect, hyaline and annellate. Conidia dark olivaceous with 3-9 pseudosepta not constricted at septa, cylindrical or curved, convexoconcave, ends blunt, truncate, measuring 21-59 × 9 – 16μ. Spores liberated in mass.

On dried stems and leaf sheaths of an unknown grass, Tanda Falls. Mirzapur (U.P.) 23-8-1958 (M. Shukla).

Type specimen deposited in the I.M.I. Herb. No. 74895 and isotype in the type collection of the I.A.R.I., New Delhi, No. 27535.

ACKNOWLEDGEMENTS : The author is indebted to Dr. R.Y. Roy, Prof. R. Misra, Department of Botany, Banaras Hindu University; Dr. J.C.F. Hopkins, Messrs C. Booth and Sutton, Commonwealth Mycological Institute, Surrey, England, who have been of help and source of encouragement. He is extremely thankful to Rev. Fr. H. Santapau, Chief Botanist, Botanical Survey of India, for kindly rendering the description into Latin and to Dr. H.K. Saksena, Government Agriculture College, Kanpur, for kindly going through the manuscript.

[1]Hughes, S. J., *Can. J. Bot.*, **31** : 677-559 (1953).

[2]Ramakrishan, K. and C. V. Subramanian, *J. Madras Univ.* B. 22 : 1-65 (1952).

———

Occurrence of Stemgall Disease of Jute caused by Physoderma corchori Lingappa in West Bengal--C. R. DAS AND P. K. SEN GUPTA, State Agricultural Research Institute, Calcutta-40.—A stemgall disease of Jute (*Corchorus olitorius L.* and *C. angulatum* Lam.) caused by *Physoderma corchori* has been reported by Lingappa[1] from Banaras and this appears to be the only report of the disease. At the Jute Seed multiplication Farm, Krishanagar, West Bengal, *P. corchori* causing gall formation on the stems was first observed in 1960 on *C. olitorius*. The symptoms were also observed during subsequent years 1961-63 on the same Farm. Field observations during last four years showed that *C. olitorius* was affected and not *C. capsularis*. Infection was high, practically all the plants were affected where the land was somewhat water-logged.

The symptoms were first noticed when the plants were 8 to 10 inches high, with the formation of small green galls on lower portion of the stem, just above the ground level. These galls gradually increased in size and turned dark brown in colour and became cracked. Several galls later coalesced to form a bigger raised area. These were noted up to a height of 7 cm. from the ground level. In some cases a few small galls were also observed on the upper portion of the stem, but no gall formations could be found on the leaf lamina as observed by Lingappa[1]. The infected plants did not succumb as a result of the disease itself but during storm or heavy winds the plants got broken at the basal infected regions.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The galls have been found to be full of resting sporangia. The resting sporangia are globose to subspherical with a circular depression on one side where the operculum is situated; smooth, dark brown in colour and measure 19.8-33.0μ (average 29.7μ). The diameters of the resting sporangia are slightly larger than those given by Lingappa (19.0-30.0μ, average 24.0μ). The resting sporangia germinate after 36 to 48 hours in tap water by protrusion of the endosporangium by pushing aside the operculum.

Microtome sections of the galls showed the presence of resting sporangia in abundance in the cortical region. These could, however, not be noticed in the xylem and pith cells. The cells when filled up with resting sporangia become hypertrophied.

Further studies with the disease are under progress.

ACKNOWLEDGEMENT: The authors wish to thank Dr. S. K. Mukherjee, Mycologist, Government of West Bengal for his interest and for going through the manuscript.

[1]Lingappa, B. T. *Mycologia*, **47** : 109-121, (1955).

———

Some fern inhabiting Cercospora species from India—G. LALL, J. N. KAPOOR AND R. L. MUNJAL, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—In this communication, three species of *Cercospora* inhabiting different ferns in India are being reported. One of them *C. lygodiicola*, is new, the other two: *Cercospora phyllitidis* Hume and *Cercospora pteridis* Siemaszko, have been observed for the first time in India. The specimens have been deposited in the *Herbarium Cryptogamae Indiae Orientalis*.

Cercospora lygodiicola spec. nov.

Foliorum maculae fuliginosae, pallidiores ad margines, amphigenae, irregulares, 2-15 mm. diam., saepe totam paginam folioli operientes; stromata constantia a cellulis paucis ve nulla; conidiophora fasciculata terna vel quaterna, nonnumquam solitaria, olivaceo-brunnea infra, subhyalina ad apicem, sparse septata, non ramosa, semel bisve geniculata, 16-47 $\times$ 4-5μ; conidia anguste obclavata, subhyalina, indistincte multiseptata, recta vel paulum curvata, subtruncata ad basin, acuta ad apicem 25-128 $\times$ 3-4μ.

Leaf spots black, irregular, some times involving the whole leaflet, 2 to 15 mm. in diameter. Fruiting amphigenous; stromata lacking or consist of only a few cells; fascicles not dense, one to many, spreading. Conidiophores olivaceous brown below and pale above, sparingly septate, unbranched, 1-2 geniculate, irregular in width, 16-47 $\times$ 4-5μ. Conidia narrowly obclavate, subhyaline, indistinctly multiseptate, straight to curved, base subtruncate, tip subacute to acute, 25-128 $\times$ 3-4μ (Fig. 1).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

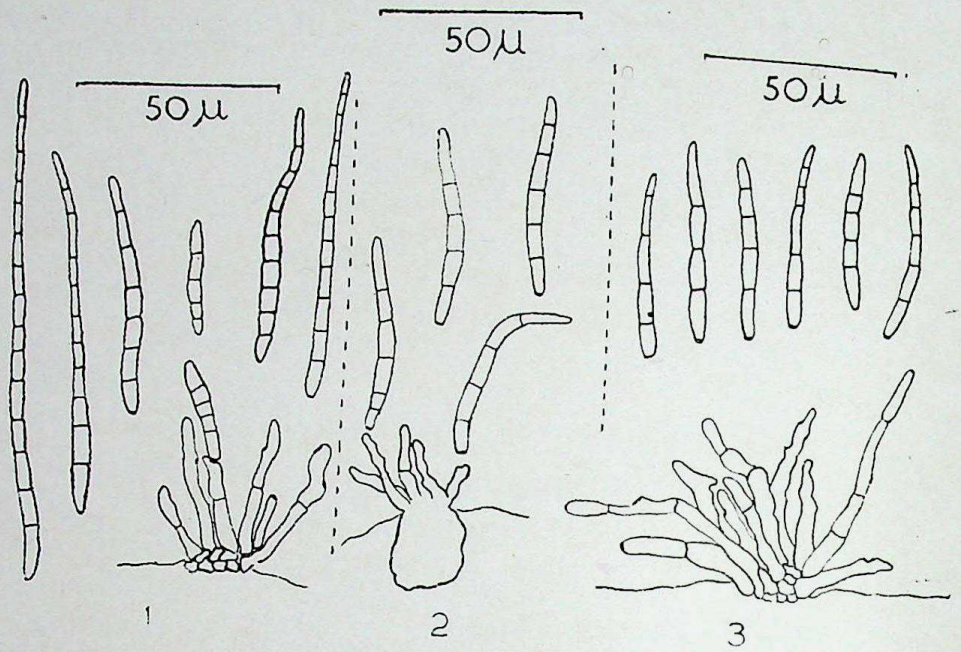


Fig. 1 *Cercospora lygodiicola*
Fig. 2 *C. phyllitidis*
Fig. 3 *C. pteridis*

On living leaves of *Lygodium japonicum* (Thbg.) Sw. (Polypodiaceae), Manali, Kulu, Punjab, November 9, 1962, V. S. Sharma, H.C.I.O. No. 28172, *Type*.

C. lygodii Sawada is recorded on this host genus from Formosa [*Formosa Agr. Res. Inst. Report.* **87** : 83, (1944)]. The specimen described above is quite distinct morphologically in all essential characters and is, therefore, being described as new species.

2. *Cercospora phyllitidis* Hume, *Bull. Torrey bot. Cl.* **27** : 577 (1900).

The fungus produces dirty white, elongated spots on the upper surface of the pinnae which are delimited by veins. The spots are tan-coloured on the lower surface. In severe cases, the spots coalesce. The spots are 2-20 mm. in diameter. The conidiophores arise in loose fascicles from dark brown erumpent stroma and are sometimes once geniculate, measuring 15-25 × 4-5μ. The conidia are obclavate, subhyaline, slightly curved, 3-7 septate and measure 45-70 × 3-4μ (Fig. 2).

On living leaves of *Pteris biaurita* L. (Polypodiaceae), Kalimpong, W. Bengal, April 1956, J. N. Gupta, H. C. I. O. No. 28171.

3. *Cercospora pteridis* Siemasvko, *Arch. Nauk. Biol. Towarz. Nauk. Warszaw*, **1** (14) : 57 (1923).

Spots on the pinnae are irregularly circular, grey in colour and measure 2-6 mm. in diameter. Fructification is mostly in the upper surface. The conidiophores are olivaceous below and paler above, geniculate, and 31-60 × 3-5μ in size. The conidia are subhyaline, cylindric, 0-5 septate, and measure 28-58 × 3-4μ (Fig. 3).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

On living leaves of *Pteridium* sp. (Polypodiaceae), I. A. R. I., New Delhi, February 2, 1963, G. Lall and V. S. Sharma, H. C. I. O. No. 28173.

Sincere thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, for his keen interest and encouragement; to the Forest Botanist, F. R. I., Dehra Dun, for the identification of the ferns; to Rev. Father Dr. H. Santapau for the latin translation of the diagnosis and to Mr. B. P. Agarwal, artist, for help in the line drawings.

Maintenance of fungi on synthetic sponge—N. S. SUBBA RAO, Division of Microbiology, Indian Agricultural Research Institute, New Delhi.—Synthetic sponge is used in making a variety of articles such as bath sponge, fancy purses, dolls and is also used as packing material involving delicate instruments. It occurred to the author whether it could be used to cultivate fungi in test tubes as a routine laboratory procedure since the material provides an inert and porous substratum for the spread of the organisms. The synthetic sponge obtained from packing material was washed well and cut in the shape of agar slopes. They were inserted into the bottom of test tubes, moistened with enough Richard's synthetic liquid medium (3-5 ml.) and autoclaved at 15 lbs. pressure for 20 minutes. The following fungi were inoculated : *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Aspergillus niger* and *Helminthosporium oryzae*. It was observed that the organisms grew well and fungi like *A. niger* and *H. oryzae* sporulated well. After 3-4 weeks incubation, either 3 ml. of fresh liquid medium was delivered into the slopes by means of a pipette or the organisms transferred to fresh slopes. The use of synthetic sponge-slopes for routine maintenance of fungi has the following features : (1) the use of agar-agar is not necessary, (2) the spongy nature of the material affords abundant aeration and increases the surface area for the ramifications of the fungal mycelia, (3) It has possibilities for culturing other microorganisms like bacteria, actinomycetes and micro-algae and (4) the material stands autoclaving, does not get denatured physically and is available on the market. It has been envisaged that culturing of fungi on discs of the material would facilitate easy and uniform inoculations of disease causing soil-borne pathogens, thus ensuring proper inoculum potential both *in vivo* and *in vitro*. A detailed study of the several ways in which the material could be used is under progress and will be reported in due course.

I thank Dr. W. V. B. Sundara Rao, Head of the division of Microbiology, I.A.R.I. and Dr. B. P. Pal, Director of the institute for their interest in the study.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Review

C.M.I. descriptions of pathogenic fungi and bacteria. Set 2, Nos. 11-20 by A. C. Hayward & J. M. Waterston, issued by the Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, England, Price 5s 0d. per set.

Sheets 11 to 20 provide abridged information about 10 important bacterial plant diseases and their pathogens. The information pertains to description of the causal bacterium, its host range and the disease incited, geographical distribution, disease transmission and additional notes on general information about the disease. In the end selected and some of the recent references are provided. The sheets should undoubtedly prove of value to research workers, teachers and students of Plant Pathology, Drs. A. C. Hayward and J. M. Waterston have evidently taken considerable pains in reviewing and pooling the information in a precise manner.

D. N. Srivastava.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CIBA Pesticides

Dimecron – a systemic insecticide, based on Phosphamidon, is effective against sucking, chewing and mining insects. It is water soluble, odourless, does not taint and most crops are free from residues ten days after treatment.

Nuvan – an insecticide with extremely potent fumigant and contact action against household and storage pests as well as against chewing and mining caterpillars on field crops.

Thiocron – an acaricide cum insecticide of low toxicity with specific action against mites.

Cuman – an organic fungicide with excellent rain resistance, suitable for the control of diseases on fruit trees and field crops.

Cosan – a wettable sulphur preparation of colloidal particle size for the control of powdery mildew on fruit crops and against mites.

Copramat – a fungicide, based on a combination of Copper-oxychloride and ziram for the control of diseases on field crops.

Stocks now available in India
For detailed information, please write to
CIBA of India Limited, Pesticides Division, P. O. Box [illegible] Bombay 1

IWT-[illegible]310 (A)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(*Contd. from 2nd cover*)

Three new bacterial diseases of plants from Maharashtra—*L. Moniz and V. P. Bhide* 149

Evaluation of certain fungicides for the control of Rhizoctonia bataticola (Taub.) Butler and Sclerotium rolfsii Sacc.—*Dharam Vir, K. D. Pathak and S. B. Mathur* ... 154

Effect of spore age and culture media on the degree of infection by Ustilago avenae—*Avdhesh Narain* 157

Efficacy of different fungicides-VIII : Field trials for the control of covered smut of barley [Ustilago hordei) (Pers.) Lager.]—*J. S. Grewal and Dharam Vir* ... 162

Sorodiscus radicicolus Cook, a new record for India—*M. S. Ghemawat* 165

Pathogenicity and varietal reaction of paddy to Sclerotium oryzae Catt. in Bihar—*A. P. Misra and Abu Mohammad* 168

Seed rot of Hevea brasiliensis caused by Botryodiplodia theobromae Pat.—*S. N. S. Srivastava* 172

Phytopathological Notes

A New Stilbospora species from India—*M. Shukla* ... 178

Occurrence of Stemgall disease of jute caused by Physoderma corchori Lingappa in West Bengal—*C. R. Das and P. K. Sen Gupta* 180

Some fern-inhabiting Cercospora species from India—*G. Lall, J. N. Kapoor and R. L. Munjal* 181

Maintenance of fungi on synthetic sponge—*N. S. Subba Rao* ... 183

Review 184

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	36.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1964

President	... ...	S. SINHA
Immediate Past President	... ...	T. S. SADASIVAN
Vice-President	... ...	N. PRASAD
Secretary-Treasurer	... ...	S. P. RAYCHAUDHURI
Joint Secretary	... ...	D. SURYANARAYANA

COUNCILLORS

Northern Zone	... ...	B. L. RENFRO
Mid-Eastern Zone	... ...	G. S. VERMA
Eastern Zone	... ...	U. N. MOHANTY
Central Zone	... ...	B. P. PATIL
Western Zone	... ...	K. G. NEMA
Southern Zone	... ...	S. B. LAL

EDITORIAL BOARD FOR 1964

R. PRASADA

Editor-in-Chief

S. P. RAYCHAUDHURI
M. J. THIRUMALACHAR
K. S. BHARGAVA
G. RANGASWAMY
KISHAN SINGH
D. N. SRIVASTAVA
M. M. PAYAK

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

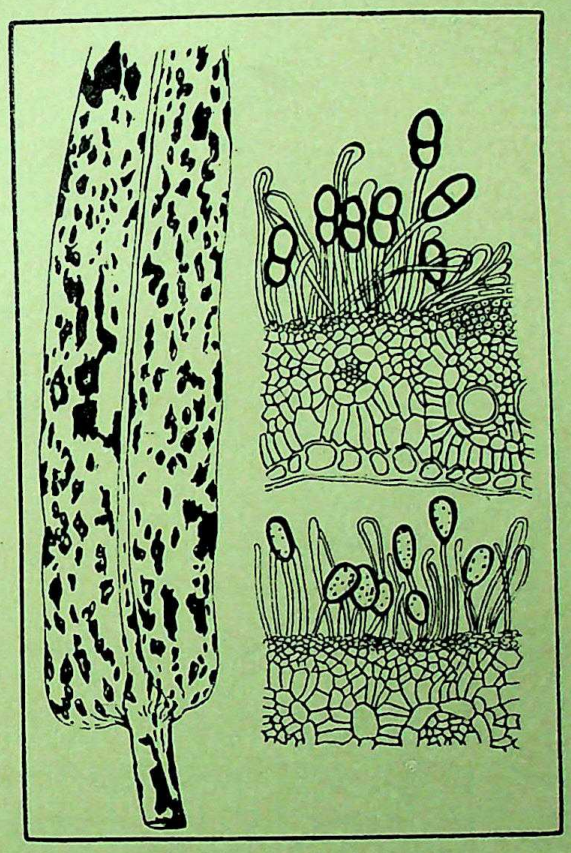
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVII **1964** *NUMBER 3*



PUBLISHED FOR

THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Dr. Jonas J. Christensen 186

A new leaf spot disease of Jowar in India—*Mahendra Pal and D. Suryanarayana* 188

Studies in Pectic enzymes of parasitic fungi—V. PECTIC ENZYMES PRODUCED BY PENICILLIUM EXPANSUM LINK—*S. C. Gupta and G. S. Rautela* 191

Studies on the survival of plant pathogens added to the soil —III. ON FOUR PHYTOPATHOGENIC BACTERIAL SPECIES—*G. Rangaswami and V. Thirunavukarasu* 202

A preliminary list of microfungi associated with the decaying stems of Triticum vulgare L. and Andropogon sorghum L.—*S. P. Lal and A. S. Yadav* 208

Studies on the population of Heterodera avenae Wollen. causing Molya disease of wheat and barley in Rajasthan —*Kishan Singh and Gopal Swarup* 212

Pycnidial stage of Hendersonula toruloidea on twigs of Psidium guajava in artificial culture—*R. L. Mathur and R. D. Singh* 216

Induced saltations in Helminthosporium oryzae Breda de Haan—*A. P. Misra and A. K. Chatterjee* ... 218

The Myxomycetes of Calcutta suburbs—*Asok Kumar Kar* ... 222

Enzymic studies of certain wood destroying fungi—*D. K. Saha* ... 224

Fungal microflora of seeds of Jowar (Sorghum vulgare) its role in reduced emergence and vigour of seedling and control—*R. L. Mathur and S. P. Sehgal* 227

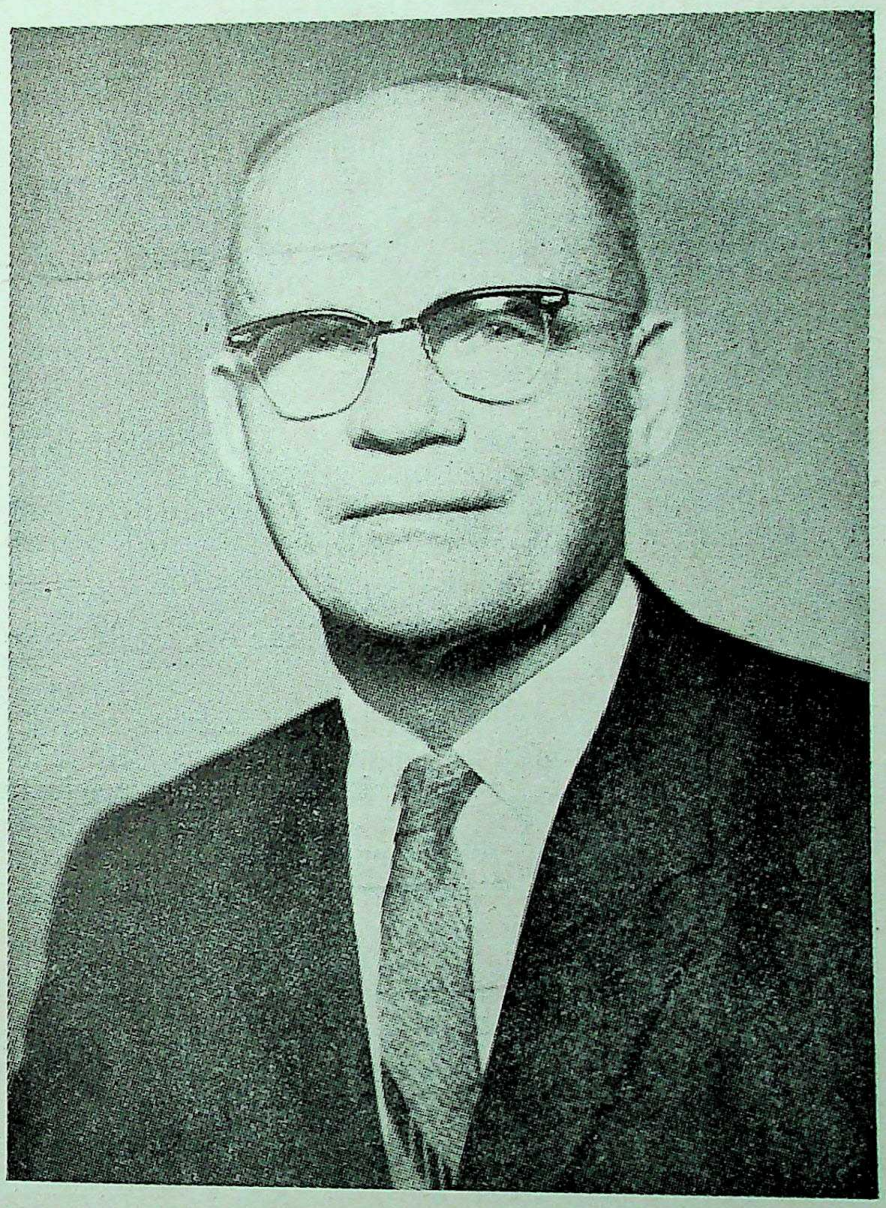
Presence of amino acids and Indole compounds in the root-knot affected plants—*G. Rangaswami and M. Balasubramanian* 234

Preliminary screening of wheat varieties against loose smut by 'Embryo test'—*R. L. Munjal and M. S. Chatrath* ... 238

(*Contd. on 3rd cover*)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



DR. JONAS J. CHRISTENSEN
1892—1964

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Dr. JONAS J. CHRISTENSEN

Dr. Jonas J. Christensen, Professor Emeritus of Plant Pathology at the University of Minnesota, born in 1892, died at St. Paul (Minnesota) on June 20, 1964. He had returned only a few days before from Denmark, where he had gone to deliver some lectures. In his passing, the University has lost one of its greatest scholars. His many students throughout the World have lost a beloved teacher and a trusted friend.

Dr. Christensen, 'Chris' as we called him, graduated in 1921 and took his M.S. degree in 1922 and the Ph. D. degree from the University of Minnesota in 1925. He started his career as a rural school teacher in Mercer, North Dakota in 1915, and was Sergent in the Medical corps during the First World War. After a couple of years as Field Agent in the Federal Horticultural Board, and State Potato Inspector, he joined as Instructor in Agriculture at the University of Minnesota in 1920, where he continued till his retirement.

He was appointed Asstt. Professor of Plant Pathology in 1925 and Professor in 1938 and rose to the position of Head of the Department of Plant Pathology and Botany in 1953 and continued in that capacity till his retirement in 1961, when he was made Professor Emeritus.

He was also Agent (Part time) of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S.D.A.

Dr. Christensen was noted for his pioneering researches in Cereal Pathology, and genetic variability in plant pathogens. He had an enthusiasm for science which was expressed in stimulating, original and evocative teaching. He inspired his students and colleagues and gave of himself without reservation. He combined the highest ideals of scholarship with a first hand understanding of the practical needs of agriculture.

Dr. Christensen won several honours. He won the Caleb Dorr Prize when senior in College in 1920, and was member of the Alpha Zeta, Gamma, Alpha and the Gamma Sigma Delta and Vice President of the Minnesota Chapter of the latter in 1943.

During 1929-30 he was awarded the Guggenheim Memorial Fellowship for study at the University of Berlin, Germany, and other European countries. In 1950, he was visiting expert in Japan and later also visited the South American countries including Brazil, Argentina, Peru, Ecuador, Colombia and Chile, and Mexico.

He won the Elvin Charles Stakman Medal for excellence in researches on Cereal diseases in 1959 and the distinguished award of Sigma X and the Outstanding Achievement Award of the Minnesota Chapter of Sigma XI in 1963.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Besides, he earned honours of the Biological Club (University of Minnesota), the Crop Protection Institute, the Flax Institute of the U.S., among others.

He was elected President of the American Phytopathological Society in 1944 and of the Sigma XI in 1949. Besides, he was member of several scientific societies including the Indian Phytopathological Society.

He helped also the Rockcfeller Foundation Programme in Mexico and was consultant to the Advisory Panel on Biological and Chemical Warfare, in the office of the Secretary of Defence, besides being a collaborator of the Cooperative Rust Laboratory of the U.S.D.A.

He suffered from a severe coronary occlusion sometime in May 1963. The attack on June 20 this year was a serious one and he could not survive. There is a vacuum in the Plant Pathological world, which is hard to fill.

Department of Mycology and Plant Pathology, A. P. MISRA
Bihar Agricultural College,
Sabour (Bhagalpur).

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A NEW LEAF SPOT DISEASE OF JOWAR IN INDIA*

MAHENDRA PAL AND D. SURYANARAYANA

(Accepted for publication September 5, 1964)

Recently in January, 1963 a leaf spot disease new to India was observed by the writers on *jowar* (*Sorghum vulgare* Pers.) at the Indian Agricultural Research Institute, New Delhi. The spots were mostly confined to the mid-rib regions. A fungus identified as *Helminthosporium rostratum* Drechs. (=*Bipolaris rostrata* Shoemaker) was isolated from these lesions.

H. rostratum was first described by Drechsler (1923) from dry leaves of *Eragrostis major* Host. at Washington. It has a wide host range being reported on wheat by Putterill (1954), on *jowar* by Kenneth (1958), on sudan grass by Tarr (1957), on rice by Chattopadhyay and Das Gupta (1959) and on *bajra* (*Pennisetum typhoides* Stapf. and Hubb.) by Luttrell (1954). Young, Lefebvre and Johnson (1947) stated that infection of the fungus is more severe on maize than on *jowar* in U. S. A. Agarwal and Shinkhede (1959) studied the physiology of the fungus isolated from foot-rot affected wheat plants.

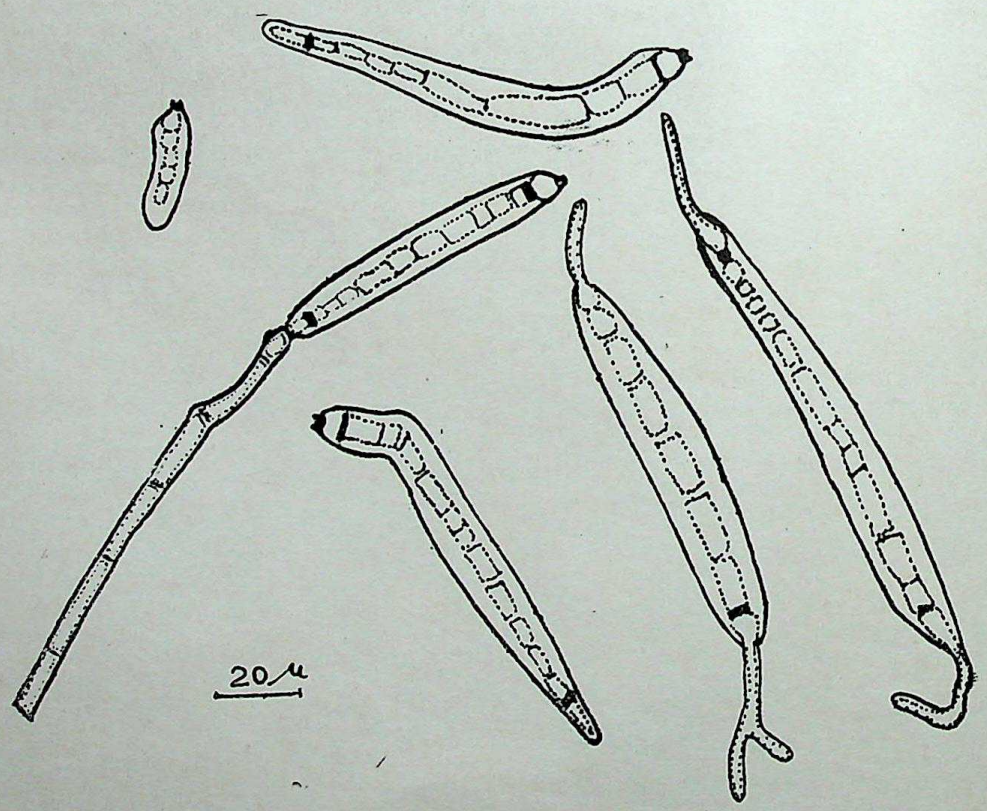


Fig. 1. Camera lucida drawings of conidiophores and conidia of *H. rostratum*.

*The paper is based on part of a thesis submitted by the senior author for the award of M. Sc. degree of I. A. R. I.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Conidiophores of the fungus are dark olivaceous and they emerge singly or in groups of 2-3 from stomata. Direct emergence of the conidiophores from epidermis was however, not noted. Conidiophores are upto 7-septate and measure 6.84 × 136.80 μ. Conidia are dark, olivaceous, straight or more or less curved, often very long and somewhat wider just below the apex and taper moderately, towards both the ends. They are typically of the rostrate type with the rostrum at the apex while the base is somewhat rounded, (Fig. 1). Conidia are 8.5-11.97 × 36-129.96 μ (mean 10.5 × 113.6 μ with 14 septa). They are 4-21 septate with the first and the last septum being somewhat darker and thicker. Conidial dimensions are more or less the same as described by Whitehead and Calvert (1959) in U. S. A. They reported conidia upto 23-septate. Conidia produced on artificially inoculated plants are smaller.

Spores were germinated in distilled water, tap water, 2 per cent glucose, 2 per cent sucrose and jowar leaf extract. Spores from 10 day-old cultures germinated after 4 hours and the maximum germination of 100 per cent was obtained in all the media tried except in distilled water where it was slightly lower (95 per cent). Spores from 3 month old cultures never gave more than 65 per cent germination and in older spores germination was mostly unipolar.

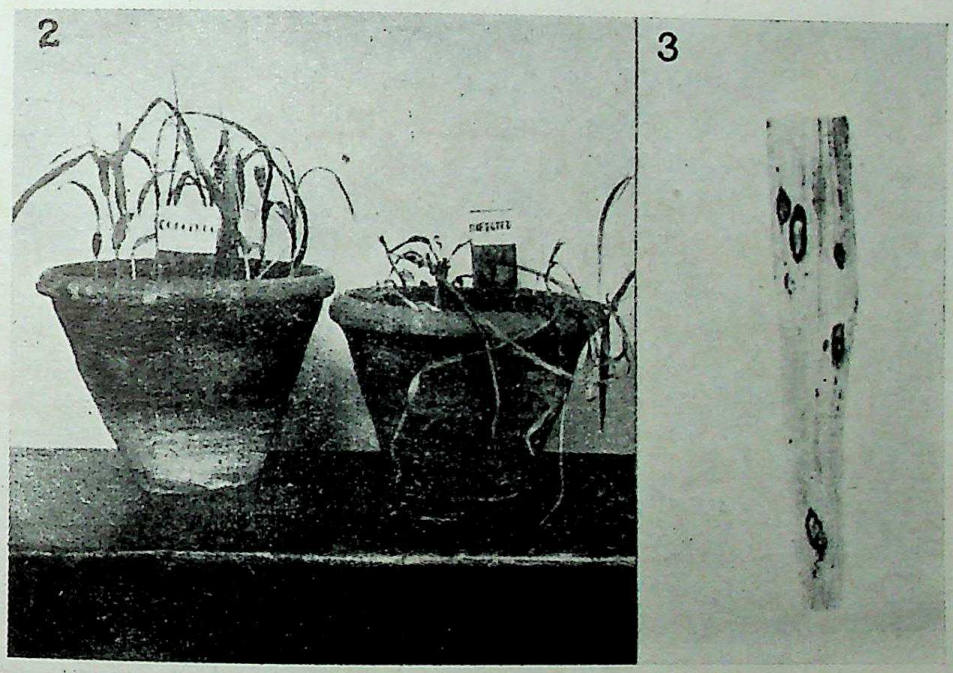


Fig. 2. Healthy and diseased seedlings of *Jowar*, the latter artificially inoculated with *H. rostratum*.

Fig. 3. Part of artificially inoculated *Jowar* leaf showing spots caused by *H. rostratum*.

Two week-old seedlings of *jowar* (variety M. 35-1), maize (Ganga 1) and of *bajra* (variety, Pusa Moti) were raised in 4″ pots and were inoculated by spraying an aqueous spore suspension with an atomizer. After inoculation, the seedlings were kept in glass cases for 36 hours. A few uninoculated seedlings were also kept under similar condition as

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

control. Small, oval to elongated, grey to straw-coloured spots measuring 5-10 mm. in length appeared on the leaves after the third day of inoculation, (Fig. 2). In *jowar* and *bajra* the spots remained separate while in maize they coalesced readily involving more or less a large area of the leaves, which resulted in their drying up eventually. The control seedlings remained healthy in every case.

The percentages of infected plants of *bajra*, *jowar* and maize obtained in these tests are : 16,100 and 92 respectively. This clearly shows that *bajra* is less susceptible than *jowar* and maize to attacks of this pathogen. Slightly older seedlings (4 week old) of *jowar* were also tested and they got only 70 per cent infection as against 100 per cent in the case of 2 week-old seedlings and this indicates that *jowar* seedlings become more resistant as they grow older.

ACKNOWLEDGEMENTS : The writers are indebted to Dr. B.L. Chona Head of the Division of Mycology and Plant Pathology, for encouragement and for providing necessary facilities for carrying out this work. The senior author also wishes to express his gratitude to Rockefeller Foundation for having given him an assistantship and other help for carrying out this investigation.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

AGARWAL, G. P. AND C. G. SHINKHEDE (1959) Physiologic studies of *Helminthosporium rostratum* Drechs. *Phyton*, **13** : 45-54.

CHATTOPADHYAY, S. B. AND C. DAS GUPTA, (1959) *Helminthosporium rostratum* Drechs. on rice in India. *Plant Dis. Reptr.*, **43** : 1241-1244.

DRECHSLER, C. (1923) Some graminicolous species of *Helminthosporium*. *J. agric. Res.*, **24** : 641-740.

KENNETH, R. (1958) Contribution to the knowledge of *Helminthosporium* flora on Graminae in Israel. *Bull. Res. Israel Sect. D*, **6** : 191-210.

LUTTRELL, E. S. (1954) Approaches to the classification of *Helminthosporium* species. *Plant Dis. Reptr. Suppl.*, **228** : 111-113.

PUTTERILL, K. M. (1954) Some graminicolous species of *Helminthosporium* and *Curvularia* occurring in S. Africa. *Bothalia*, **6** : 347-378.

SHOEMAKER, R. A. (1959) Nomenclature of *Drechslera* and *Bipolaris* grass parasites segregated from *Helminthosporium*. *Can. J. Bot.*, 37 : 879-887.

TARR, S. A. J. (1957) Rep. Sect. Bot. Pl. Path. In *Ann. Rept.. Res. Div. Min. Agric. Sudan*, 1955-1956.

WHITEHEAD, M. D. AND O. H. CALVERT; (1959) *Helminthosporium rostratum* ear rot of corn and leaf spot of thirteen grass hosts. *Phytopathology*, 49 : 817-820,

YOUNG, G. Y., C. L. LEFEBVRE, AND A. G. JOHNSON, (1947) *Helminthosporium rostratum* on corn, sorghum and pearl millet. *Phytopathology*, 37 : 180-183.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES IN PECTIC ENZYMES OF PARASITIC FUNGI

V. PECTIC ENZYMES PRODUCED BY *PENICILLIUM EXPANSUM* LINK

S. C. GUPTA AND G. S. RAUTELA

(Accepted for publication August 4, 1964)

A number of fruits have been found to be parasitised by *Penicillium expansum* in India (Singh, 1941; Singh and Grewal, 1953; Gupta and Pandey, 1959). In earlier studies, Gupta (1960a and b) reported that this fungus secreted protopectinase enzyme on several fruits which were attacked by it and on several other natural media. In a later communication it was reported that *P. expansum* produced quite active protopectinase and polygalacturonase enzymes on a specific synthetic medium which contained pectin (Gupta, 1960c). It was also found that ammonium nitrate was a better source of nitrogen than asparagin or sodium nitrate for the production of the two enzymes. The secretion of pectin-esterase was, however, found to be of negligible activity on synthetic medium.

Protopectinase enzyme has also been reported to be produced by *P. ehrlichii* (Ehrlich, 1932), *P. chrysogenum* (Phaff, 1947) and *P. citrinum* (Roboz and Kertesz, 1948), and polygalacturonase has been described from *P. chrysogenum* (Phaff, 1947), *P. citrinum* (Roboz and Kertesz, 1948) and *P. notatum* (Lulla and Johar, 1953). However, pectin-esterase has been reported *in vitro* from only *P. chrysogenum* (Phaff, 1947) and *in vivo* from *P. expansum* (Cole, 1958). Earlier information regarding the properties of protopectinase and polygalacturonase secreted by *Penicillium* is very scanty.

In the present work the authors have studied the effect of different nitrogen sources on the production of protopectinase and polygalacturonase enzymes by *Penicillium expansum* and the various factors affecting the activity of the pectic enzymes thus produced.

MATERIAL AND METHODS : The strain of *Penicillium expansum* used in this work was an isolate from infected apples obtained from the stock cultures of Botany Department, D.S.B. Government College, Naini Tal. The cultures were maintained on potato-dextrose agar slopes at 25°C.

For the preparation of pectic enzymes the fungus was grown on 15 ml. of synthetic liquid culture medium taken in each flat bottle of 12 fluid oz. capacity. Most commonly used culture medium (glucose-pectin-nitrate medium) contained 0.5 per cent glucose, 1.0 per cent pectin, 0.28 per cent ammonium nitrate, 0.05 per cent magnesium sulphate and 0.1 per cent potassium di-hydrogen phosphate. The sterilization was done by autoclaving at 15 lbs pressure for 20 minutes.

At the end of four days incubation period fungal-mat was removed from each culture bottle, washed with warm water, dried at 70°C and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

then weighed. The filtrate was centrifuged at 5,000 r.p.m. for 5 minutes and the clear transparent liquid was used for assaying different enzymes.

Protopectinase (PP) activity was assayed by potato disc method originally used by Brown (1915) and detailed by Gupta (1960c). The percentage of PP was calculated by the following formula :—

$$\frac{1.000}{MT},$$

where MT indicated the maceration time in minutes of the standard potato discs when kept in the enzyme preparation of which the activity was to be calculated.

Polygalacturonase (PG) enzyme was assayed by studying the loss of viscosity of 1 per cent sodium polypectate solution (details given by Gupta, 1960c). When the viscosity of sodium polypectate solution fell to the viscosity of distilled water in 1 minute, it was assumed that PG was 100 per cent active.

No pectin-esterase (PE) activity was assayed as it was found to be very low by Gupta (1960c).

All the pH measurements were taken on a Cambridge pH meter.

EXPERIMENTAL : (i) *Effect of different nitrogen sources on enzyme production* : Ammonium nitrate was replaced with different organic and inorganic nitrogenous compounds in the culture medium on the basis of equal available nitrogen. Activity of PP, PG and dry weight of the fungal mycelium in different sets of experiments were taken and the results are shown in Table 1.

TABLE 1 : Effect of nitrogen sources on enzyme production

S. No.	Medium	Initial pH	Final pH	percent of PP	percent of PG	Dry wt. in mg
1.	M+Asparagin	3.5	5.8	20.0	49.3	88
2.	M+Amm. nitrate	3.6	3.7	91.0	96.2	54
3.	M+Amm. phosphate	3.8	3.6	45.4	91.6	70
4.	M+Amm. sulphate	3.8	3.6	*	92.3	56
5.	M+Amm. chloride	3.8	3.6	*	78.3	53
6.	M+Amm. tartrate	5.0	4.8	15.4	47.8	85
7.	M+Amm. oxalate	5.0	3.6	40.0	94.4	67
8.	M+Amm. hepta-molibdate	5.2	4.9	*	19.9	16
9.	M+Sodium nitrate	3.4	6.8	20.0	76.9	71
10	M+Pot. nitrate	3.6	5.1	15.5	67.8	90
11.	M+Mag. nitrate	3.4	4.9	21.3	63.0	66
12.	M+Cal. nitrate	3 4	4.7	16.6	***	75
13.	M+Alum. nitrate	3.4	3.8	8.3	81.8	10
14.	M+Uranium nitrate	3.3	—	—	—	**
15.	M+Hippuric acid	2.7	2.9	19.2	50.0	45
16.	M+Urea	6.3	7.0	31.3	62.0	65

M – Medium without nitrogen source.
*—Activity less than 0.5 per cent.
**—No growth.
***—Sodium polypectate solution got coagulated.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

From the results it was clear that ammonium nitrate was the best nitrogen source for the secretion of both PP and PG. While nitrate salts in general were better than ammonium salts for the growth, pectic enzyme secretion was observed to be more with the latter. Ammonium sulphate and ammonium chloride did not support the secretion of PP, while very active PG was secreted by the fungus on these two salts. This finding not only supports the view that PP and PG are two different enzymes but also that the two can be produced by the fungus independent of each other.

(ii) *Effect of pH on the activity of pectic enzymes :* For studying the effect of pH on PP and PG activity, the enzyme preparation was obtained by the method described above and its activity was tested over a range of 2.0—8.0 pH. The activity of PG could not be measured at pH lower than 4.0 as further lowering of the pH caused jelly formation in the sodium polypectate solution.

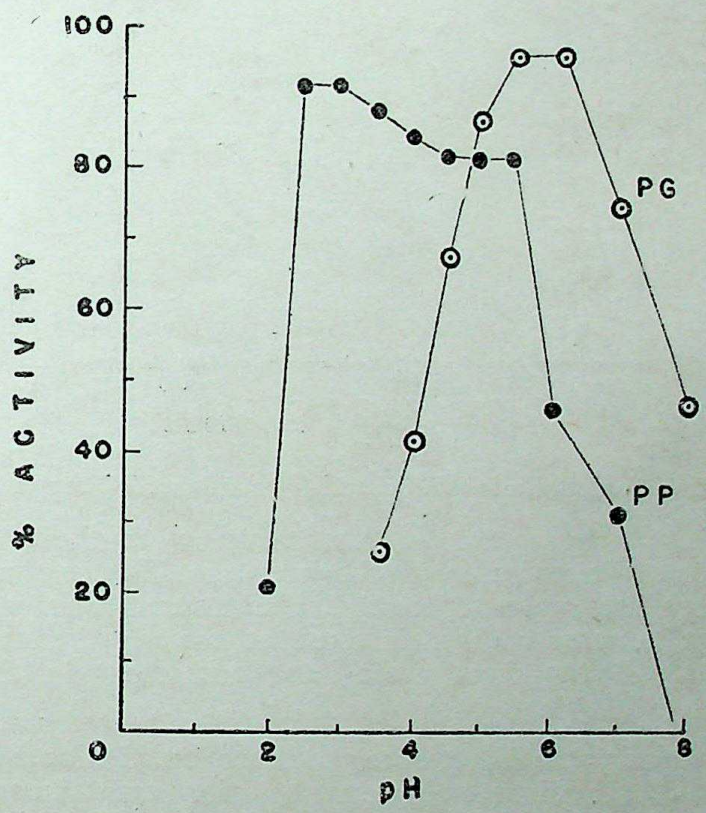


Fig. 1. Effect of pH on the activity of PP and PG enzymes.

Results shown in Fig. 1 indicate that PP was most active at lower pH values (most active at 2.5-3.0 pH) while PG was more active at comparatively higher pH values (most active at 5.5-6.2 pH). However, the activity of both PP and PG rapidly fell down on the alkaline side as the activity of PP at 8.0 pH was practically nil and that of PG was only 30 per cent at the same pH.

(iii) *Effect of dilution* : Enzyme preparation was diluted with distilled water and pH of the various dilutions was brought to the same level.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It was found that the activity of both the enzymes decreased with the increased dilution. The activity of PP responded quickly to any variation in its dilution while that of PG showed no marked reduction in its activity upto 70 per cent dilution of the enzyme (Fig. 2). Dilution of the enzyme preparation to two times caused 50 per cent reduction in PP activity while it caused only 11.2 per cent loss in the activity of PG enzyme. In both, PP and PG, a marked fall in their activity was observed beyond 90 per cent dilution of the enzyme preparation.

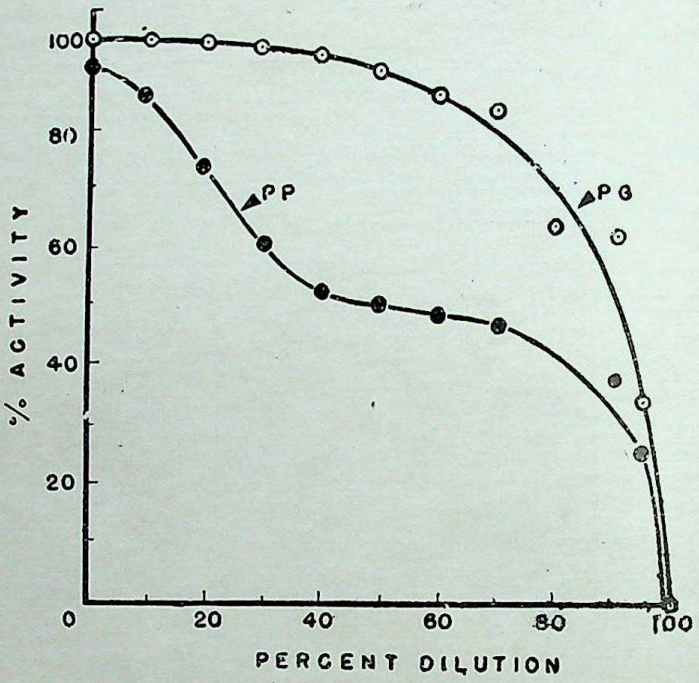


Fig. 2. Effect of dilution on the activity of PP and PG enzymes.

(iv) *Effect of temperature on the activity of enzymes* : For determining the effect of temperature on the activity of PP, enzyme preparation and potato discs were separately taken in small thin walled glass tubes and kept in water bath maintained at the required temperature for 5 minutes. Potato discs were later immersed in the enzyme preparation .

For determining the effect of temperature on the activity of PG, enzyme preparation, sodium polypectate solution and viscometer were separately kept at the required temperature in a water bath for 5 minutes and later the activity of the enzyme was noted by the method described earlier.

The pH and other factors were kept constant in all the experiments. It was concluded from the results (Fig. 3) that the activity of PP enzyme increased moderately when the temperature was raised from 20° to 25°C, while a marked acceleration occurred from 30°C onwards upto 45°C. A decline in the activity of the enzyme was noted when it was tested at a temperature of 55°C or above.

The increase in the activity of PG enzyme, with the increase in temperature was more gradual in comparison to PP enzyme. However,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

a decline in the activity of the enzyme was noted from 50°C onwards (Fig. 3).

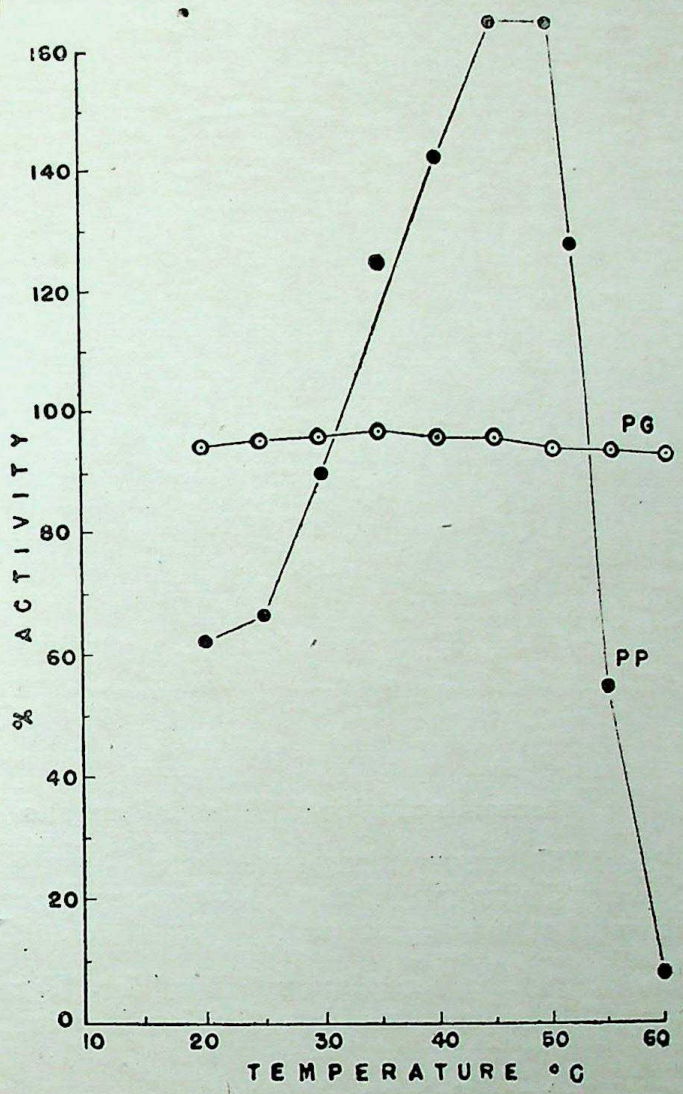


Fig. 3. Activity of PP and PG enzymes at different temperatures.

(v) *Thermal inactivation* : Enzymes were brought to 6.2 pH before the temperature treatments. Thin-walled glass tubes were first immersed in water bath at the required temperature for 3 minutes, the enzyme was poured in the tube and stirred for 10 minutes. The tube with the enzyme was then transferred to ice cold water to cool it at once. The activities of PP and PG were tested at 30°C and 6.2 pH. The results are shown in Fig. 4.

From the results it was clear that there was steady loss in the activity of PP when the enzyme was treated at temperatures higher than 40°C. The enzymes got more inactivated at 60° or 80°C than at 65° or 90°C. However, a complete loss of the activity of PP was observed when the enzyme was treated at 80°-85°C but its activity was better when it was treated at 90° or 100°C for ten minutes.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

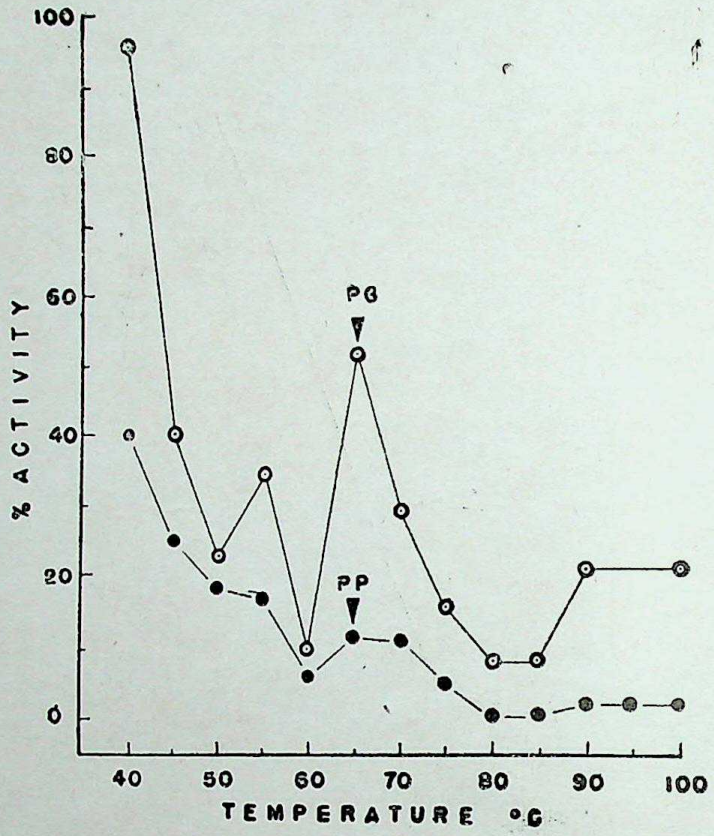


Fig. 4. Thermal inactivation of PP and PG enzymes

The activity of PG enzyme also got affected by the temperature treatment in the way similar to PP, but the former enzyme could not be completely inactivated at any temperature.

The enzyme preparation was also autoclaved for 10 minutes separately at 5 lbs. and 10 lbs. pressures, but complete inactivation of either PP or PG enzyme was not observed.

(vi) *Effect of drying* : Only 10 ml. of the enzyme preparation was dried under vacuum inside a desiccator. The dried powder was then dissolved in 10 ml. of distilled water and this solution was tested for the activities of PP and PG. The results obtained have been shown in Table 2.

TABLE 2 : Effect of d ying on the activity of enzymes

Particulars of enzyme	per cent of PP	per cent of PG
Normal enzyme	91.0	96.2
After drying	79.0	95.0

It was clear from the results that when the enzyme was dried under vacuum and again made to the original volume by dissolving it in distilled water, PP and PG lost their activity by 12 and 1.2 per cent respectively.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(vii) *Effect of purification :* Purification of the enzyme preparation was done by dialysis and precipitation with alcohol and acetone.

For dialysis 10 ml of enzyme preparation was enclosed in a parchment paper along with a drop of toluene and was placed in a beaker filled with distilled water for 24 hours at 5°C. The water was frequently changed.

For precipitation of the enzyme with alcohol, 20 ml. of absolute alcohol was added to 5 ml. of enzyme preparation in test tube, corked and placed at 0°C for 24 hours. Similarly 20 ml. of acetone was mixed with 5 ml of enzyme and placed at 0°C for 24 hours. In both the cases the precipitate was collected separately by centrifuging at 5,000 r.p.m. for 15 minutes, dissolved in 5 ml of distilled water and assayed for the two enzymes.

It is clear from the results (Fig. 5) that dialysis retarded the activity of both the enzymes drastically. That the reduction in the activity was not due to its storage, is clear from Figs. 6 and 7. Precipitation with either acetone or alcohol also retarded the activity of PP and PG but to a lesser extent. The activity of PP enzyme was more affected by precipitation than that of PG.

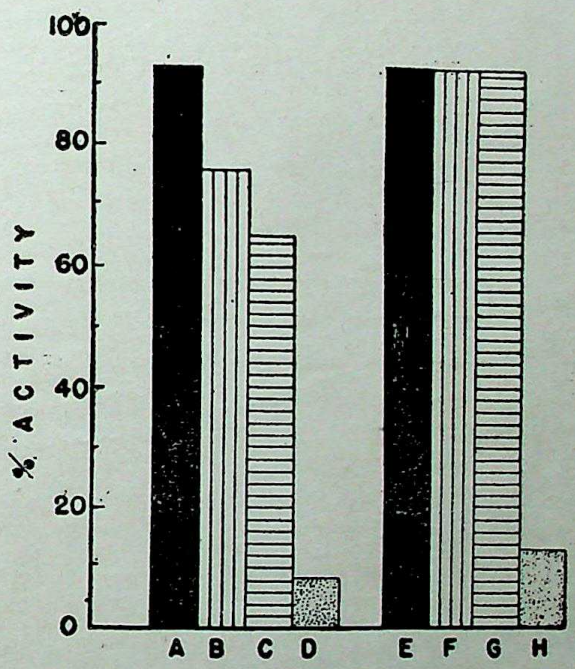


Fig. 5. Effect of purification on the PP and PG enzymes. Activity of PP enzyme : before purification-A ; after precipitation with acetone-B ; after precipitation with alcohol-C ; after dialysis-D. Activity of PG enzyme : before purification-E ; after precipitation with acetone-F ; after precipitation with alcohol-G ; after dialysis-H.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

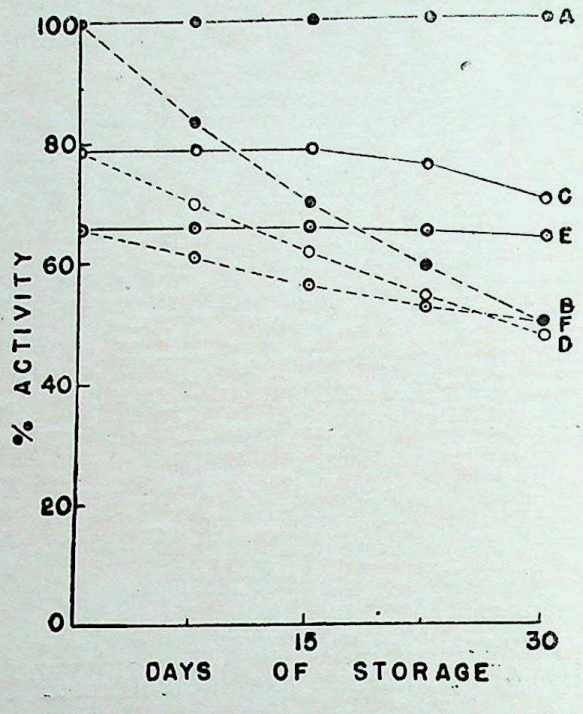


Fig. 6. Activity of PP enzyme when stored as such at 0°C (A) and at 25°C (B); stored after drying at 0°C (C) and at 25°C (D) and stored after precipitation at 0°C (E) and at 25°C (F).

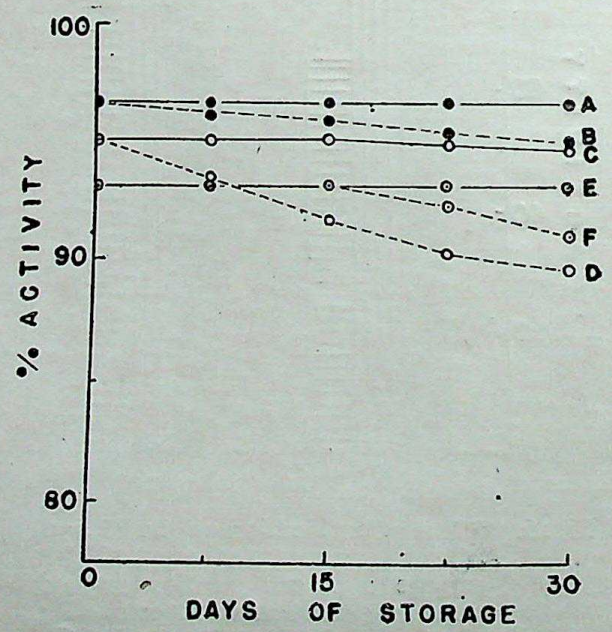


Fig. 7. Activity of PG enzyme when stored as such at 0°C (A) and at 25°C (B); stored after drying at 0°C (C) and at 25°C (D); stored after precipitation at 0°C (E) and 25°C (F).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(viii) *Effect of storage of enzyme :* In order to find out if the enzyme preparation could be stored in the laboratory without loss in the activity of different pectic enzymes, it was kept under the following conditions :—

(a) The crude enzyme preparation under toluene at 0°C and 25°C.

(b) In powder form after drying the enzyme preparation at 0°C and 25°C.

(c) The enzyme was precipitated with 75 per cent ethanol, the precipitate air dried and then stored at 0°C and 25°C.

The activity of the two enzymes was tested at regular intervals over a period of 30 days. Results are shown in Figs. 6 and 7.

It was concluded from the results (Fig. 6) that there was practically no loss in the activity of PP when the enzyme was stored at 0°C for 30 days under any of the 3 conditions while at 25°C, 50 per cent of its activity was reduced under condition (a), 41 per cent under condition (b) and 26 per cent under condition (c). However, PG enzyme was found to be more stable when the enzyme preparation was stored either at 0°C or 25°C under any of the three conditions mentioned above (Fig. 7).

DISCUSSION : The evidence obtained in this work is in general agreement with that obtained by other workers (Foster, 1949) that ammonium nitrogen is better than the nitrate nitrogen for the secretion of pectic enzymes. Though Singh and Wood (1956), in the case of *Fusarium moniliforme*, showed that asparagin was a better source of nitrogen than ammonium nitrogen, Gupta (1960c) and the present authors definitely found that asparagin nitrogen was inferior to ammonium nitrate for the production of pectic enzymes by *P. expansum.*

It seems to be strange why pectic enzymes became more inactive when kept at 60° and 80°C for ten minutes than kept for the same period at higher temperatures. Though the authors are unable to suggest any reason to explain it but such a phenomenon has been reported with other pathogens also (Wood, 1955; Kamal and Wood, 1956).

Due to unclarified chemical definition of protopectin and inaccurate methods for the measurement of the activity of protopectinase (PP), Kertesz (1951) questioned the existence of protopectinase enzyme. As PP enzyme has often been found in association of polygalacturonase (PG) enzyme with similar physical properties, it led to the assumption that PP and PG are identical enzymes. However, Wood and Gupta (1958) showed that while *Pythium debaryanum* produced macerating (PP) enzyme, there was no trace of PG or PE in the same preparation thus contradicted that PP and PG are the identical enzymes. In the present work also the authors found that *Penicillium expansum* can be made to produce PP or PG, independent of each other, in quite an active state by altering the nitrogen source in the medium (Table 1). The optimum pH for the activity of PP and enzymes was also found to be different. Thus the authors feel that though the macerating enzyme (PP) can not be defined accurately yet, it seems to be different from that of PG.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SUMMARY

Production of protopectinase and polygalacturonase enzymes by *Penicillium expansum* on synthetic media and the physical properties of the two enzymes were studied.

In general, ammonium nitrogen has been found to be better for pectic enzyme production than nitrate nitrogen. The optimum pH for the activity of PP was found to be between 2.5-3.0 while for PG between 5.5-6.2. When the activity of the two enzymes was tested at different temperatures, both of them showed more activity with the increase in temperature till the inactivation started. Heating of the enzyme preparation at 60° or 80°C caused much inactivation than heating at 65° or 90°C. Complete inactivation of enzymes could not be obtained even after autoclaving the enzyme preparation for 10 minutes at 10 lbs. pressure. The two enzymes could be stored either in crude form, dried form or after precipitation for several weeks at 0°C without the loss in their activity. It has been discussed that PP and PG are not identical enzymes.

ACKNOWLEDGEMENTS : The authors are grateful to the U.P. Scientific Research Committee, Allahabad, for the financial aid and to the Sunkist Growers, California, U.S.A., for supplying the various pectic materials used in this work.

Botany Department,
K. N. Government College,
Gyanpur (Varanasi).

REFERENCES

BROWN, W. (1915) Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. *Ann. Bot., Lond.*, **29** : 313-348.

COLE, M. (1958) A comparative study of the rots of apples caused by different fungi. *Ph. D. Thesis*, University of London, England (quoted from Wood, 1960).

GUPTA, S. C. (1960a) The secretion of protopectinase enzyme and the growth of *Penicillium expansum* on natural media. *Proc. 47th Indian Sci. Cong.*, **Part III** : 335.

——————— (1960b) Protopectinase enzyme secretion by *Penicillium expansum* Link in the infected fruits. *Curr. Sci.*, **29** : 146.

——————— (1960c) Studies in pectic enzymes of parasitic fungi. III. Secretion of pectic enzymes by *Penicillium expansum* Link. *Proc. Indian Acad. Sci.*, **B 52** : 103-109.

——————— AND D. K. PANDEY (1959) Market diseases of fruits and vegetables in Kumaon. III. *Penicillium* rot of fruits. *J. biol. Sci.* **2** : 6-8.

EHRLICH, F. (1932) *Biochem. Z.*, **251** : 204 (quoted from Kertesz, 1951).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FOSTER, J. W. (1949) *Chemical activities of fungi.* Academic Press, Inc., N.Y.

KAMAL, M. AND R. K. S. WOOD (1956) Pectic enzymes secreted by *Verticillium dahliae* and their role in the development of wilt disease of cotton. *Ann. appl. Biol.*, **44** : 322-340.

KERTESZ, Z. I. (1951) *The pectic substances.* Inter Science, Inc, N.Y.

LULLA, B. S. AND D. S. JOHAR (1953) *Penicillium notatum* as a source of fungal pectinase. *Curr. Sci.*, **22** : 79-80.

PHAFF, H. J. (1947) *Arch. Biochem.*, **13** : 67 (quoted from Kertesz, 1951).

ROBOZ, E. AND Z. I. KERTESZ (1948) Unpublished work (quoted from Kertesz, 1951).

SINGH, B. AND J. S. GREWAL (1953) Soft rot of pears (*Pyrus communis* Linn.). *Proc. nat. Acad. Sci., India.* **B32** : 153-156.

SINGH, R. K. AND R. K. S. WOOD (1956) Studies in the physiology of parasitism. XXI. The production and properties of pectic enzymes secreted by *Fusarium moniliforme* Sheldon. *Ann. Bot., Lond.*, (*N.S.*) **20** : 89-103.

SINGH, U. B. (1941) The soft rot of apple fruit in Kumaon. *Indian J. agric. Sci.*, **11** : 902-905.

WOOD, R. K. S. (1955) Pectic enzymes secreted by pathogens and their role in plant infection. Mechanism in microbial pathogenicity, *5th Symposium Soc. Gen. Microbiol.*, 263-293.

WOOD, R. K. S. AND S. C. GUPTA (1958) Studies in the physiology of parasitism. XXV. Some properties of the pectic enzymes secreted by *Pythium debaryanum. Ann. Bot., Lond.*, (N.S.) **25** : 309-319.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE SURVIVAL OF PLANT PATHOGENS ADDED TO THE SOIL

III. ON FOUR PHYTOPATHOGENIC BACTERIAL SPECIES

G. RANGASWAMI AND V. THIRUNAVUKARASU

(Accepted for publication August 5, 1964)

In the earlier communications from this laboratory the survival of some plant pathogenic microorganisms in soil, and their inhibition by antagonistic organisms were reported (Rangaswami and Prasad, 1961; Rangaswami and Ramalingam, 1962; Rangaswami and Ethiraj, 1963). In the present paper the survival of four species of plant pathogenic bacteria in soil when added as cell suspension or with the respective host tissue is reported.

MATERIAL AND METHODS : Fresh isolates of *Pseudomonas solanacearum* E. F. Smith from brown rot of potato, *Xanthomonas maydis* Rangaswami *et al.* from leaf spot of corn, *X. rubrilineans* (Lee *et al.*) Starr and Burkholder from leaf stripe of sugarcane and *X. rubrisorghi* Rangaswami *et al.* from red leaf blotch of sorghum were isolated and used in these studies. The procedure followed for examining the survival of bacteria in soil, when added as cell suspension or with the host tissue, was the same as used by Rangaswami and Ethiraj (1963). The soil used in these studies was obtained from the Annamalai University Experimental Farm. It was clayey loam, with a pH of 6.9, organic matter of 1.48 per cent, total nitrogen 0.08 per cent, total P_2O_5 0.12 per cent, available P_2O_5 0.007 per cent, K_2O 3.1 per cent, and C_aO 0.93 per cent. It contained on an average 25.4 million microorganisms per gm of soil on moisture free basis at 0-3 inch layer, 16.6 million at 3 to 6 inch layer and 12.4 million at 6 to 12 inch layer.

To examine the influence of population of the pathogen on the survival period, serial dilutions of the cell suspension were prepared in sterile distilled water, and the suspensions were added in equal quantities to soil contained in one lb bottles. Population changes in the soil were analysed at weekly intervals by the dilution plate method, using soil extract agar for bacteria and actinomycetes and potato dextrose agar for the fungal populations, care being taken to obtain representative soils amples. Plating of each sample was done in triplicates and the average for each treatment obtained.

To find out the population of saprophytic microorganisms carried on the surface of infected host tissues samples of uninfected and infected host tissues were collected at random from the fields. The samples were cut into 1 sq. cm. bits, each bit was placed in 100 ml sterile distilled water in 250 ml Erlenmeyer flasks, shaken thoroughly and 1 ml portions of the suspension plated in nutrient or potato dextrose agar. The dry weight of the plant tissue was obtained in the usual manner and the total microbial population per gram of host tissue, on dry weight basis, and per sq. cm of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Survival of bacterial pathogens in soil when added as cell suspension
(Data represent average of triplicates under each treatment)

Treatment/Pathogen	A*	B**	A	B	A	B	A	B	A	B
In sterile soil :										
Ps.solanacearum	21.5	250	2.2	250	0.2	250	0.02	250	0.002	250
X. maydis	2.76	84	0.28	98	0.03	91	0.003	84	0.0003	84
X. rubrilineans	12.90	84	1.29	98	0.13	91	0.013	91	0.0013	84
X. rubrisorghi	31.40	112	3.10	112	0.31	112	0.03	112	0.003	105
In unsterile soil :										
Ps. solanacearum	21.5	250	2.2	250	0.2	250	0.02	250	0.002	250
X. maydis	2.76	56	0.28	28	0.03	14	0.003	28	0.0003	3
X. rubrilineans	12.90	56	1.29	49	0.13	42	0.013	3	0.0013	3
X. rubrisorghi	31.40	63	3.10	35	0.31	28	0.03	14	0.003	3

*A=Initial bacterial population ($\times 10^6$) added to soil (per g.).
**B=Survival period in days.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the surface of host tissue were calculated. There were quadruplicates under each set of treatments and the data obtained were bulked and the average for each treatment obtained. All these studies were carried out by incubating the bottled soils and microbial cultures at room temperature (26 to 30°C).

RESULTS : *Survival in soil when added as cell suspension* : The results on survival of the four bacterial species when added as cell suspension to sterile and unsterile soils are summarized in Table 1.

Ps. solanacearum survived for over 250 days in both sterile and unsterile soil when added as cell suspension, irrespective of the variations in the quantity of the initial populations added. In the cases of *X. maydis*, *X. rubrilineans* and *X. rubrisorghi* the survival period in sterile soil ranged from 84 to 112 days as against 3 to 63 days in unsterile soil. The initial bacterial population added to sterile soil seemed to have little influence on the survival period whereas when added to unsterile soil, the larger initial population persisted for relatively longer period than the smaller populations.

Survival in soil when added with host tissues : The results on the survival of plant pathogens when added with the respective host tissues are summarized in Table 2.

TABLE 2 : Survival of the bacterial pathogens in soil when added with host tissue (the data represent average of triplicates under each treatment)

Pathogen	Survival period in days			
	Sterilized soil		Unsterilized soil	
	Surface sterilized host bit	Surface unsterilized host bit	Surface sterilized host bit	Surface unsterilized host bit
Ps. solanacearum	250	250	250	250
X. maydis	90	75	60	60
X. rubrilineans	75	60	45	45
X. rubrisorghi	90	60	45	45

These results indicate that *Ps. solanacearum*, carried in potato tubers, could survive for over 250 days in sterile and unsterile soils, whether the tubers were surface sterilized or not before burying in the soil. In the cases of the three species of *Xanthomonas*, the survival period varied from 45 to 90 days. When the host tissues were surface sterilized to kill the externally-borne contaminants, the pathogen inside seemed to survive for more days in sterile soil, whereas there was no such variation in unsterile soil.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Estimation of Microbial Load on Host Tissues : In the above experiment there were indications for the interference of microbial contaminants on the host tissues with the survival period of the pathogens. In order to estimate the total microbial load on the host tissues, studies were made as per the details given under Materials and Methods. The results are summarized in Table 3.

TABLE 3 : Microbial load on the leaves of sorghum and sugarcane (the data represent the average of quadruplicates under each treatment)

Host leaf	Microbial population					
	per sq. cm. of leaf surface			per gram of leaf on dry wt. basis		
	B*	A**	F***	B	A	F
Sorghum leaf :						
healthy	4	105	90	1750	52300	25060
infected with *X. rubrisorghi*	26	102	85	10920	42840	35700
Sugarcane leaf :						
healthy	8	96	96	2957	36890	36890
infected with *X. rubrilineans*	14	99	92	5740	40590	37720

*B=Bacteria.

**A=Actinomycetes.

***F=Fungi.

The data indicate that the host tissues carried more actinomycetes and fungi than bacteria per sq. cm. or per gram of leaf tissue. There were relatively more bacteria on the diseased leaf bits than on healthy bits, which might be because of presence of the pathogenic bacteria on the host bits.

DISCUSSION : The results obtained in the present studies indicate that the species of *Xanthomonas* declined rapidly in soil, whereas *Ps. solanacearum* could survive for over 250 days. The period of survival of three *Xanthomonas* spp., when added as cell suspension, was affected by the quantity of inoculum, there being a proportional reduction of the survival period in unsterile soil with the reductions in the cell population added. These results are similar to the findings of Semenuik and Henry (1960) on *Ophiobolus graminis*, *Helminthosporium sativum* and *Fusarium culmorum*. The quicker suppression of *Xanthomonas* spp. in unsterile soil than in sterile soil, and when added with the host tissues than as cell suspension, seemed to confirm the earlier reports from this

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

laboratory (Rangaswami and Prasad 1961; Rangaswami and Ramalingam 1961, 1962; Rangaswami and Ethiraj 1963). The possible action of surface contaminants in suppressing the pathogenic bacteria in the host tissue is also indicated in the present studies. It is interesting to note that the actinomycetes and fungi were the predominant organisms on the host tissues rather than bacteria. The activation of contaminants and or the soil saprophytes due to the addition of host tissues to the sterile or unsterile soil may at least contribute in part towards the reductions in survival periods of the pathogens in the host tissues.

Ps. solanacearum survived for a longer period than the other three species, indicating thereby that it could overwinter from season to season either as free cells or inside the host tissues. These findings are in line with the findings of Martin (1939) and Staples (1957) on the same organism. In the present studies the incubation temperature of the soil ranged from 26 to 30°C, which is rather narrow. Further studies are required to examine the influence of varying soil temperatures on the survival period of the bacterium.

Summary

Studies were made on the survival in sterile and unsterile soils of four plant pathogenic bacteria, viz., *Pseudomonas solanacearum* E. F., Smith causing brown rot of potato, *Xanthomonas maydis* Rangaswami *et al* causing leaf spot of corn, *X. rubrilineans* (Lee *et al.*) Starr and Burkholder causing leaf stripe of sugarcane and *X. rubrisorghi* Rangaswami *et al.* causing red leaf blotch of sorghum. *Ps. solanacearum* was found capable of surviving in soil, both when introduced as water suspension and with infected host tissue, for over 250 days, to permit the soil to carry over the pathogen form season to season. The other three bacteria were able to survive only for a period of 84 to 112 days in sterile siol and for 3 to 63 days in unsterilized soil and for still lesser periods when added with their respective host tissues. When different quantities of cell suspensions of the three species of *Xanthomonas* were added to unsterile soil, there was a progressive decline in the population, but not in sterile soil. There were indications for the suppressive role of the microbial load of the host leaves on the bacterial pathogens carried in them.

Microbiology Laboratory,
Department of Agriculture,
Annamalai University,
Annamalainagar, Madras.

References

Martin, J. P. (1939) Pathology. *Rep. Hawaii Sugar Expt. Sta.*, 1938 : 30-38.

Rangaswami, and S. Ethiraj (1962) Antibiotic production by *Streptomyces* sp. in unamended soil. *Phytopathology* **52** : 989-992.

Rangaswami, G. and S. Ethiraj (1963) Studies on the survival of plant pathogens added to the soil. II. *Helminthosporium sacchari*, *H. sativum* and *H. turcicum*. *Indian Phytopath.* **16** : 10-14.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RANGASWAMI, G. and N. N. PRASAD (1961) Studies on the survival of plant pathogens added to the soil. I. *Fusarium* spp. and *Xanthomonas cassiae*. *Indian Phytopath.*, **14** : 83-87.

RANGASWAMI, G. and M. RAMALINGAM (1961) Studies on the survival of plant pathogenic bacteria in sterilized and unsterilized soil. *Indian J. Microbiol.* **1** : 153-156.

RANGASWAMI, G. and M. RAMALINGAM (1962) Survival of *Helminthosporium oryzae* in soil and its inhibition by *Bacillus mycoides*. *Phytopathology*, **52** : 347-351.

SEMENUIK, G. and A. W. HENRY (1960) Relative decline of *Ophiobolus graminis*, *Helminthosporium sativum* and *Fusarium culmorum* in the soil. *Can. J. Plant Sci.*, **40** : 288-293.

STAPLES, R. R. (1957) Report of the Department of Research and Specialist Services (Southern Rhodesia). *Rep. Minist. Agric. Rhod.*, Nyasaland, 1958 : 7-86.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A PRELIMINARY LIST OF MICROFUNGI ASSOCIATED WITH THE DECAYING STEMS OF TRITICUM VULGARE L. AND ANDROPOGON SORGHUM L.

S. P. LAL AND A. S. YADAV

(Accepted for publication August 20, 1964)

Synecological studies of fungi colonizing the above ground plant remains have yielded a large number of microfungi including many interesting species, [Webster (1956-57), Gremmen (1957), Hudson and Webster (1958), Yadav (1960a and 1960b)]. The authors while studying the ecology of fungi colonizing the decaying stems of *T. vulgare* and *A. sorghum* observed a large number of microfungi which are listed here. Their synecology, physiology and systematics as well as their role in the decomposition of the above ground plant debris are being studied in detail and shall be published later.

MATERIAL AND METHODS: The senescent stems of *Andropogon sorghum* and *Triticum vulgare* were cut from the soil level and stacked in piles in November, 1961 and March, 1962 within the college campus. The methods of study of fungus colonizers of the stems were the same as those followed by Webster (1956, 1957) and Yadav (1960b). Ten samples from each stem-substrate were taken out from each pile every month for study. This was followed for a period of 15 months.

OBSERVATIONS: Among the fungi observed on the decaying stems of both the hosts as mentioned below, none of the colonizers belong to Phycomycetes. Another important point is that the majority of the colonizers are Fungi Imperfecti.

TABLE 1 : Non-sporulating fungi associated with decaying stems of *A. sorghum* and *T. vulgare*.

Fungi	*A. sorghum*	*T. vulgare*
Choanephora sp.	+	+
Pullularia pullulans	+	+
Cephalosporium sp.	+	—
Helicosporium sp.	+	—
Fusarium moniliforme	+	—
Botryodiplodia theobromae	—	+
Geotrichum sp.	—	+
Rhodotorula sp.	—	+
Trichoderma lignorum	—	+

N.B. +Present.
—Absent.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It is noteworthy that some fungi, as shown in Table 1, occur as non-sporulating mycelia within the stems and they sporulate freely when well-washed pieces of decomposed stems infested with their mycelia are planted on the nutrient agar. The reason for non-sporulation of these fungi within the decaying stems may presumably be due to the associative effects of other members of the fungal population of decaying stems and are worth investigating.

TABLE 2 : Pioneer colonizers associated with the decaying stems of *A. sorghum* and *T. vulgare*

Fungi	*A. sorghum*	*T. vulgare*
Cladosporium herbarum	+	+
Alternaria tenuissima	+	–
A. tenuis	–	+
Curvularia lunata	+	+
Helminthosporium hawaiiense	+	+
Fusarium moniliforme	–	+
F. semitectum	+	–
Nigrospora sphaerica	+	–
Epicoccum nigrum	+	+
Paecilomyces perscicinus	+	–
Pullularia pullulans	+	+

N.B. +Present.
—Absent.

Table 2 shows the pioneer colonizers of the stems under study. Since pioneer colonizers first open the attack on the stems of ageing plants, they were suspected of having weak parasitic tendencies and were therefore, tested for their pathogenicity by inoculating them aseptically on to the freshly-cut pieces of living stems of *A. sorghum*. It was interesting to note that *A. tenuissima*, *C. herbarum*, *C. lunata*, *E. nigrum*, *F. semitectum*, *H. hawaiiense*, *P. perscicinus* and *P. pullulans*, were capable of producing infection on the green stem pieces of *A. sorghum*.

It is evident that the floristic composition of the fungus population of the decomposing stems is not similar. However, there are some fungi, common to both the types of stems. Thus it may be concluded that the decaying herbaceous stems harbour a large number of microfungi in nature and each type of these stem-substrates may have its own specific fungal association.

Following fungi were found on both the host-substrates.

ASCOMYCETES : *Metasphaeria sorghi*, *Sphaerulina phyllostachydis*,

DEUTEROMYCETES : *Alternaria tenuis*, *A. tenuissima*, *Arthrinium saccharicola*, *Bispora* sp., *Circinotrichum thevetiae*, *Cladosporium herbarum*, *Curvularia lunata*, *Cytospora* sp. 1, *Cytospora* sp. 2, *Dictyoarthrinium sacchari*, *Diplodiella* sp., *Coniothecium* sp., *Epicoccum nigrum*, *Fusarium*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

moniliforme, *Helminthosporium hawaiiense*, *H. sativum*, *Memnoniella echinata*, *Nigrospora sphaerica*, *Pithomyces sacchari*, *Spegazzinia tucumanensis*, *Sporodum atropurpureum*, *Stauronema cruciferum*, *Stagonospora* sp., *Plenodomus sorghi*, *Phoma complanata*, *Phoma* (5 unidentified spp.), *Pyrenochaeta* sp., *Tetraploa aristata*, *Torula herbarum*, *Torula* sp. and *Volutella* sp.

The following fungi were found on *A. sorghum* alone.

ASCOMYCETES : *Aspergillus niger*, *Nectriella dacrymycella*, *Glomerella cingulata*, *Gibbera* sp., *Lophiostoma* sp., and an unidentified pyrenomycete.

MYXOMYCETES : *Physarum pulcherimum*.

DEUTEROMYCETES : *Articulospora tetracladia*, *Botryodiplodia theobromae*, *Corynespora cassicola*, *Colletotrichum graminicola*, *Cytosporella carnea*, *Coniothyrium sorghi*, *Cephalothecium roseum*, *Diplodia* (sp. 4 and sp. 5), *Heliscus longibrachiatus*, *Hendersonia* sp., *Hyalostachybotrys sacchari*, *Fusarium semitectum*, *Lacellina graminicola*, *Microxyphium* sp., *Paecilomyces perscicinus*, *Periconia digitata*, *Periconiella echinochloae*, *Trichosperma* sp. —(?), *Taeniolella* sp., *Trichoderma lignorum*, *Trichobotrys effusa*, *Tetraploa* sp. and two unidentified Coelomycetes.

On decaying stems of *T. vulgare* following Deuteromycetes were met with : *Cylindrosporium* sp., *Dinemasporium graminum*, *Diplodia* (2 unidentified spp.), *Diplodina* sp., *Hormiscium* sp., *Trinacrium* sp., (?) and two other unidentified Hyphomycetes.

SUMMARY

Microfungi colonizing the decaying stems of *Triticum vulgare* and *Andropogon sorghum* have been enumerated, some of which occur only as non-sporulating mycelia. Majority of the first colonizers were found to possess weak parasitic tendency. The decomposing stems harbour a large number of fungi in nature and each type of stem-substrates may have its own specific fungal association.

ACKNOWLEDGEMENT : The authors express their sincere thanks to Drs. M. B. Ellis, C. Booth, Mr. E. J. Elphic, Mr. B. C. Sutton and Miss Stockdale of C. M. I. Kew England for helping with the identification of many of the fungi.

Thanks are also due to Principal N. S. Nagendranath of Science College, Patna and Professor R. P. Roy of University Department of Botany, Patna University for the facilities provided.

Science College,

Patna.

REFERENCES

GREMMEN, J. (1957) Microfungi decomposing organic remains of pines. *Overdruk Int. Fungus*, **27** : 34-42.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

HUDSON, H. J. AND WEBSTER, J. (1958) Succession of fungi on decaying stems of *Agropyron repens*. *Trans. Brit. mycol. Soc.*, **41** : 165-177.

WEBSTER, J. (1956) Succession of fungi on decaying cocksfoot culms I. *J. Ecol.*, **44** : 517-544.

———— (1957) Succession of fungi on decaying cocksfoot culms II. *J. Ecol.*, **45** : 1-30.

YADAV, A. S. (1960a) *Dactylella arnaudii* sp. nov. *Trans. Brit. mycol. Soc.*, **43** : 603-606.

———— (1960b) A study of fungi associated with the natural decay of the stems of *Urtica dioica L.* and *Heracleum sphondylium* L. and of their succession, *Ph. D. Thesis*, University of London.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE POPULATION OF HETERODERA AVENAE WOLLEN. CAUSING MOLYA DISEASE OF WHEAT AND BARLEY IN RAJASTHAN

KISHAN SINGH* AND GOPAL SWARUP

(Accepted for publication August 5, 1964)

Cereal root nematode causing a disease popularly known as 'Molya' is perhaps the most serious disease of wheat and barley in the light soil regions of Rajasthan. It is characterised by severe stunting of plants, poorly developed root system, poor tillering (Fig. 1) and inhibited spike emergence (Fig. 2). The disease occurs in patches of varying sizes and causes great reduction in yield of barley and wheat, particularly the former. A nematode, *Heterodera avenae* Wollenweber,

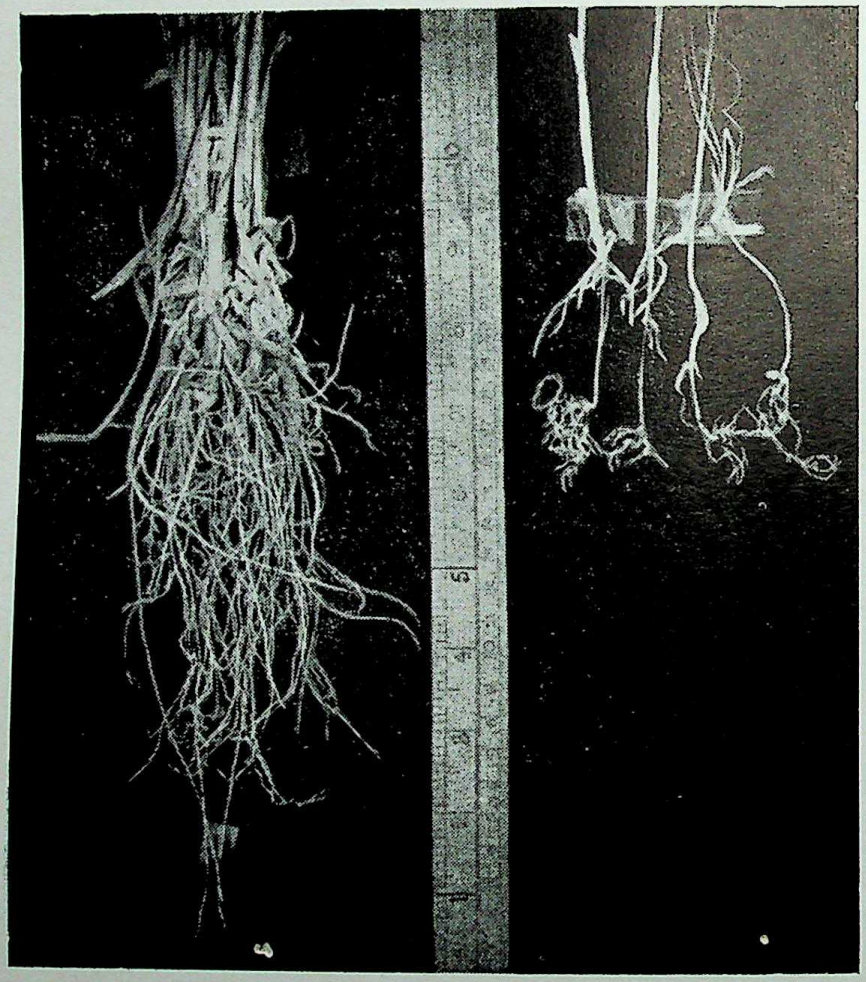


Fig. 1. Roots of a healthy (left) and diseased plants.

*Senior author at present Mycologist, Indian Institute of Sugarcane Research, Lucknow.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

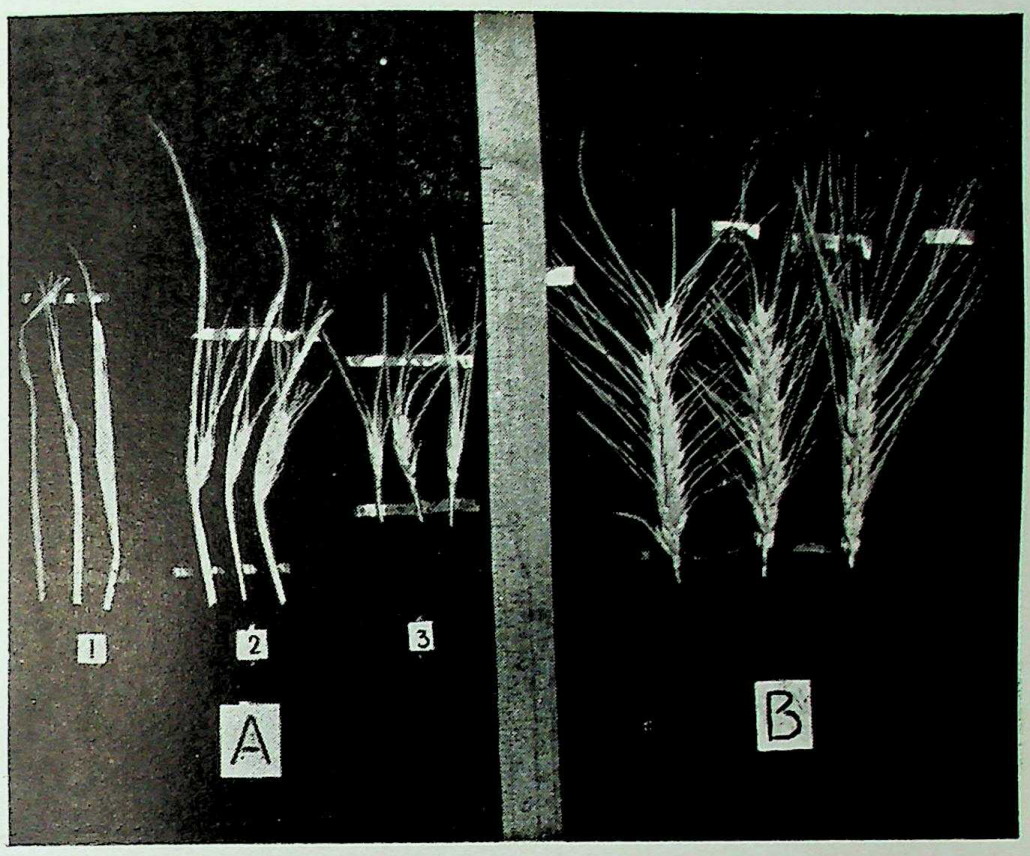


Fig. 2. (A) Spikes of diseased plants—unopened (1), partially open (2), completely open (3) ; (B) Spikes of healthy plants.

1924 (Syn. *H. major*) was established as the cause of this disease (Swarup and Singh[2]). The development of the various stages of this nematode and their correlation with the intensity of the disease is briefly described in this paper.

Composite soil and root samples were drawn at random from affected fields in different months of the season. These were analysed for the population of larvae, adult males and cysts of *H. avenae* per 1000 g. of soil in each case. The larvae were isolated by the modified Baermann funnel technique (Christie and Perry[1]). Cysts and larvae were collected on 60 and 300 mesh sieves respectively. Adult males were also collected on the latter type of sieve. Freshly-formed cysts were often found attached to roots and could not be separated from them even with a strong jet of water and therefore had to be hand-picked from the débris collected on the 60 mesh sieve.

The results of the soil analyses showed that in all cases where the the crop was diseased, a large number of larvae and cysts of this nematode were found to be present in the soil or roots. However, in cases where the crop showed no apparent symptoms of the disease these were either absent altogether or present in very small numbers. The intensity of the disease in the later part of the growing season appeared

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

to be directly correlated to the numbers of larvae early in the season and to the number of cysts towards the end in all the cases examined. Figures of average population of *H. avenae* and estimates of disease incidence are given in Table 1.

TABLE 1 : Average population of *H. avenae* in samples obtained from affected fields in various months

Time of collection	District of collection	Crop in affected field	Number of samples analysed	Average number per sample			Per cent disease incidence
				Larvae	Adult males	Cysts	
December	Jaipur	Barley	5	1363	—	1	75
	Ajmer	Wheat	2	421	—	4.5	10
January	Jaipur	Barley	6	350	129	0.2	20-60
		Wheat	1	897	20	—	30
February	Sikar	Barley	4	331	—	29.5	30-50
		Wheat	1	453	—	52	50
	Jaipur	Barley	1	553	—	48	50
March	Jaipur	Barley	6	0	—	909	40-60
April	Sikar	Barley	4	0	—	695	30-75
		Wheat	1	0	—	86	40
	Jaipur	Barley	1	0	—	821	50

The samples collected in a month were from different localities. The samples obtained in different months from a district were not necessarily from the same field.

The first symptoms of the disease appear in the crop by the end of November or early December, 3 to 4 weeks after sowing. It is at about this time that the maximum number of larvae of the nematode were found in the soil. Absence of fully developed cysts, at this stage, indicates that the young larvae came from cysts either present in the field at the time of sowing or brought into it through irrigation water or any other similar agency. The larvae enter the young roots, and establish fresh infection. This explains the gradual decrease in larval population around the roots in the middle of the season. As the roots are attacked in the early stages, their normal development is affected. New rootlets are formed but these in turn, contract fresh infection with the result that the root system becomes bunchy, a symptom typical of a diseased plant (Fig. 1).

In January, the motile nematode population around the root zones of affected plants consisted mostly of adult males. The developing females with their white posterior ends protruding out of the roots were also observed in the samples collected in January. Sex differentiation

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

in larvae takes place at about this time. The hard brown covering on the females developed mostly later in the season, although a few appeared to mature in January-February. These early maturing females are possibly responsible for the presence of the small number of young larvae collected from samples in February.

Samples collected in March and April, yielded fully developed brown cysts only. The absence of whitish females on the roots in these months indicates that either new infection ceases to take place or the larvae within the roots do not develop. At this period male nematodes were also not observed in the rhizosphere of the affected plants.

A few larvae of *H. avenae* were found in a soil sample collected from a field (village Nanura, district Jaipur) where no cereals had been raised during the preceding five years, indicating thereby that the nematodes were either brought into the field with irrigation water from some adjoining affected areas or that viable larvae were still left in old cysts. These aspects need further investigation. The role of other crops and weeds common in cultivated areas, in Rajasthan, as collateral hosts of this nematode is another aspect that needs to be studied.

SUMMARY

Monthly analyses of the soil samples from fields of barley or wheat affected with 'Molya' disease showed that a direct correlation exists between population of *H. avenae* in the soil and intensity of the disease. It appears that *H. avenae* completes one life cycle in one crop season under field conditions. Controlled studies in this respect are being conducted.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. R. S. Vasudeva, formerly Head of the Division of Mycology and Plant Pathology for helpful discussions during the course of investigations and to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for going through the manuscript critically.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

[1]Christie, J. R. and V. G. Perry. *Proc. Helm. Wash.*, **18** : 106-108 (1951).
[2]Swarup, Gopal and Kishan Singh. *Indian Phytopath.*, **14** : 127-133 (1961).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

"PYCNIDIAL STAGE OF HENDERSONULA TORULOIDEA ON TWIGS OF PSIDIUM GUAJAVA IN ARTIFICIAL CULTURE

R. L. MATHUR AND R. D. SINGH

(Accepted for publication August 20, 1964)

Since the torula stage of *H. toruloidea* Nattrass, isolated from the drying twigs of guava (*Psidium guajava* L.) was reported by the authors[1] the pycnidial stage of the fungus has also been found and is being reported in the present paper. Tubes containing potato dextrose agar slants were inoculated with the culture of the fungus, two days later, surface-sterilised pieces of guava twigs were placed and 5 ml. of sterile water added after 20 days of inoculation to maintain optimum humidity. The fungus grew profusely on the twigs giving rise to arthrospores and in about 1½ month, erumpent stromatic balls were found on the surface of the bark, and within three months the pycnidia and pycnospores of *H. toruloidea* were observed.

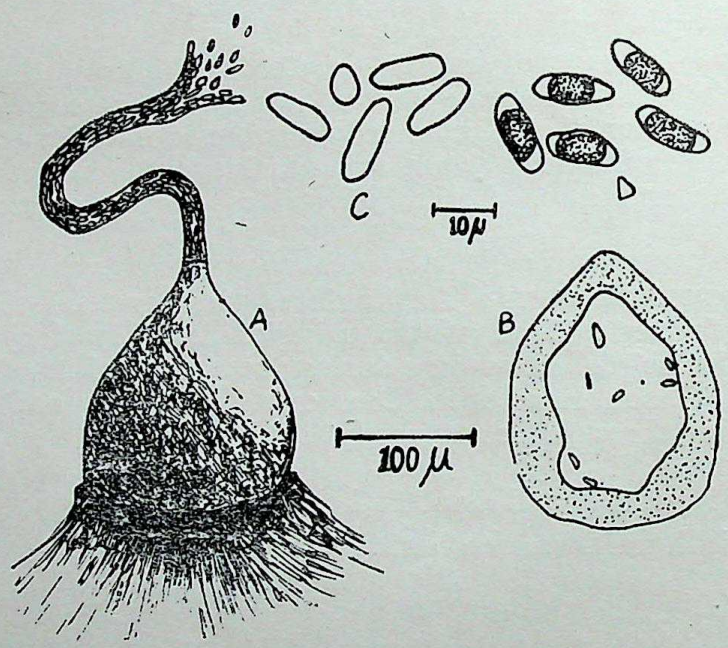


Fig. 1. *H. toruloidea*.—A : Pycnidium with cirrhus protruding from top. B : Section through pycnidium showing locule and pycnospores. C : Hyaline pycnospores. D : Dark septate pycnospores.

The pycnidia (fig. 1A) were globose to flask-shaped measuring 101-221 μ in diameter and 102-238 μ in height, one or more in a stroma; on soaking, the pycnidia produced white milky cirrhi from the top (fig. 1B) which consisted of hyaline nonseptate, globose to elongate guttulate pycnospores (fig. 1C) 2.7×14.4 μ long and 2.7 to 7.2 μ

[1]Mathur M. L. and R. D. Singh, *Curr. Sci.*, **28** : 124-25, (1959).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

broad (average 4.2 to 7.4μ). Later, the spores turned brown to black and transversely two septate ones with the central cell darker and bigger in size developed (fig. 1D); the end cells of these spores were lighter in colour and sometimes collapsed.

The pycnospores germinated readily in water the hyaline spores germinating from the ends and the coloured ones through the central cells. Both types of spores, when grown on PDA gave rise to the typical mycelium and conidia (arthrospores) of *H. toruloidea* thus establishing the link between the torula and pycnidial stages of the fungus.

The culture of *H. toruloidea* received from the Commonwealth Mycological Institute, England behaved similarly in the production of conidia and pycnidia. Dark 3-celled spores were also found in this culture which were not observed by Wilson[2].

The writers are grateful to the Director, Commonwealth Mycological Institute, Kew for the supply of culture.

Plant Pathology Section,
Department of Agriculture,
Jaipur, Rajasthan.

[2]Wilson E. E. *Phytopathology*, **29** : 124-25, (1949).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDUCED SALTATIONS IN HELMINTHOSPORIUM ORYZAE BREDA DE HAAN

A. P. MISRA AND A. K. CHATTERJEE

(Accepted for publication September 16, 1964)

Matsuura (1930) reported the formation of white sectors in *Helminthosporium oryzae* Breda de Haan at 28°C which reverted to the parent form at higher or lower temperatures. Chattopadhyay and Das Gupta (1958) and Misra and Mukherjee (1962) studied the effects of certain media and temperature on saltation in the fungus. The present investigations were made in furtherance of the above work with an isolate of the pathogen from Sabour, in order to determine the conditions which induce the production of saltants and whether these remain stable.

MATERIAL AND METHODS: The following synthetic and non-synthetic media were used in course of various experiments. Glucose peptone agar, Czapek's agar, Coon's agar, Richard's agar, potato-dextrose agar, maize meal agar, oat meal agar, and host extract agar. The media were prepared according to the standard formulae (Ainsworth, 1961; Riker and Riker, 1936). Inoculations were made with single spores of the fungus derived from a stable colony maintained on potato-dextrose agar. The inoculated plates were incubated at desired temperatures.

In order to study the effect of sodium chloride on saltation, it was incorporated in the basal Richard's agar medium, in concentrations varying from 1 to 4 percent, prior to autoclaving. Twenty ml. of the sterilized medium were poured in sterile petri plates, inoculated with single spores when sufficiently cooled and incubated at 30°C.

For determining stability of saltants in successive generations the saltants were sub-cultured for three generations, S_1 and S_2 on Richard's agar medium, S_3, S_4 and S_5 on potato dextrose agar medium (see foot notes). The parent culture was also subcultured for comparison. The first generation inoculum was derived from saltant plate consisting of single spores or mycelial tips, inoculum for second generation being derived from first. The cultures were incubated at 30°C and observations were made after six days.

RESULTS AND DISCUSSION: *Effect of media and temperature on saltations*: The media mentioned above were inoculated with single spores of the fungus. Observations after six days revealed that saltations appeared only on Richard's agar medium and in no other media. Sectors were

S_1—Saltant obtained on Richard's agar at 30°C.

S_2—Saltant obtained on Richard's agar with 3 percent sodium chloride at 30°C.

S_3, S_4 and S_5—Saltants obtained on potato-dextrose agar at 16°C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

both of patch and fan types, defined by white mycelial type in dark parental colony.

To study the effect of temperature on saltations two sets of petri plates containing 20 ml. of Richard's agar medium and potato-dextrose agar medium were inoculated with single spores and incubated at 16, 30, 32 and 35°C. Saltations were observed on both potato dextrose agar and Richard's agar at 16°C but were most common on Richard's agar. Sectors were mostly of the fan type, though patch type were also evident. At 30°C saltations were observed only on Richard's agar and on potato-dextrose agar. The saltants were less sharply defined and infrequent.

TABLE 1 : Effect of temperature on saltation in *H. oryzae*

Temperature in °C.	Media	Saltants
16	Potato-dextrose agar	6 out of 10 plates
	Richard's agar	8 out of 10 plates
30	Potato-dextrose agar	Nil.
	Richard's agar	5 out of 10 plates
32	Potato-dextrose agar	Nil.
	Richard's agar	Nil.
35	Potato-dextrose agar	Nil.
	Richard's agar	Nil.

Effect of sodium chloride on saltations : Sodium chloride at 2 and 3 percent concentrations only, induced the formation of saltants (2-3 out of 5 plates); saltants were absent at 0, 1 and 4 percent concentrations. Sectors were of patch and fan types, mostly the latter. At 1 percent concentration of the salt the margin of the colonies became irregular and its topography changed, radial corrugations developed in otherwise uniform colonies. At 4 percent concentration the growth of the colony was appreciably inhibited, no saltants formed and the mycelium remained white with slightly pinkish tinge.

Morphological characters and stability of saltants in successive generations : Parent : Mycelium variable, diameter 4.2-8.5μ, average 6.7μ, lobulations and anastomosis present, colour of mycelium hyaline when young, later turning brown, spores abundant, elliptical, brown, average size 17.2 $\times$ 10.3μ, 3-, 4-septate.

Growth of the colonies on Richard's agar medium and potato-dextrose agar medium 67.1 and 72.8 mm. respectively with abundant sporulation. On both media colonies uniform and olive green in colour, but on potato-dextrose agar more compact and tended to be darker in colour.

S_1 : Derived from fan-shaped sector, white, fluffy, alternating with black parental type, mycelium like the parent with average diameter of 6.7μ, hyaline but changing to light olive colour towards

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the base. *Spores absent*. For three successive generations on Richard's agar medium the saltant showed difference in amount of growth from the parent; average growth of the colony in first three generations 52.0, 52.3 and 56.2 mm; other variant characters like shape, margin of colony, pigmentation and lack of sporulation were lost by the third generation.

S_2 : Derived from fan-shaped, white, loose, mycelial type sector, mycelium similar to S_1 and *spores absent*. The saltant completely reverted to the parent form by the third generation.

S_3 : Derived from fan-shaped sector consisting of silvery-white submerged mycelium.

Mycelium variable, two distinct types, thin hyaline with an average diameter of 6.2μ, and thick light brown, average diameter 6.8μ, septations wide apart in the former and closer in the latter, anastomosis and lobulations present, *spores absent*. The average growth of the colony on potato-dextrose agar medium in the first three generations 85.4, 83 and 82.6 mm. successively; there was no sporulation. Quite distinct from the parent, S_3 had loose, non-compact, occasionally submerged white mycelium which tended to be olive green.

S_4 : Derived from fan-shaped sector, white, aerial, fluffy, mycelial type alternating with black parental type. *Spores absent*.

The diameter of colonies on potato-dextrose agar for three successive generations 88.2, 87.4 and 87.5 mm. respectively. Other characters similar to S_3.

S_5 : Derived from black submerged fan-shaped sector, mycelium dark olivaceous green, average diameter 8.4μ, septate, septations closer at base but well separated towards the tip. Spores dark brown, mostly 3-septate, elliptical, average size $21.4 \times 10.5\mu$.

On potato-dextrose agar the saltant showed lesser amount of growth (diameters of colonies in three successive generations were 52.8, 54.4 and 55.5 mm. (respectively) than the parent (72.6 to 72.8 mm.); sporulation similar to that of the parent, but the saltant colony had both aerial and submerged mycelium; colour of the colonies of both the parent and saltant was dark olive green but in the latter it tended to be black.

The correlation drawn by Stevens (1922) in species of *Helminthosporium* was observed in S_3, S_4 and S_5. Both the saltants S_3 and S_4 in general showed faster growth rates accompanied by paleness of colour, absence of sporulation and loose mycelium, whereas, S_5 was slow-growing, dark-coloured with abundant sporulation

The phenomenon of 'pseudomyceliolysis', as reported by Matsuura (1930) was observed in case of S_3 and S_4, in which sinking of aerial mycelium was pronouned. In contrast to the observations of Hiroe (1935), none of the saltants with 'pseudomyceliolysis' gave rise to saltants.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Behaviour of saltants on different media : S_3, S_4 and S_5 showed more or less consistent differences from the parent in growth, sporulation, pigmentation and topography in all the media tested. The remaining saltants behaved similarly to that of the parent, except, S_1 which grew faster on Richard's agar medium than the parent,

Thus it is evident that S_3 S_4 and S_5 were different from the parent, differences being consistent and quite marked, indicating thereby that they arose probably due to genetical change which was of permanent nature.

Summary

Saltations were induced in *Helminthosporium oryzae* in Richard's agar and potato-dextrose agar media at 16°C. At 30°C, saltants were observed in Richard's medium only. Sodium chloride added to Richard's agar medium at 2 and 3 percent concentrations induced the formation of saltants. The saltants formed at low temperature were of permanent nature, but others reverted to the parent form.

Department of Plant Pathology,
Bihar Agricultural College,
Sabour, (Bhagalpur).

References

AINSWORTH, G. C. (1961) *Dictionary of the Fungi*. Commonwealth Mycological Institute, Kew, Surrey.

CHATTOPADHYAY, S. B. AND C. DASGUPTA, (1958) Saltation in *Helminthosporium oryzae* Breda de Haan. *Indian Phytopath.*, **11** : 144.

HIROE, I. (1935) Experimental studies on the saltation in Fungi (Prel. Rept.) IX - on the biological character of pseudomyceliose. *Ann. Phyto. Soc. Japan*, **4** : 178-190.

MATSUURA, I. (1930) Experimental studies on the saltation in Fungi (Preliminary Rept.) I. On the saltation of *Ophiobolus miyabeanus* parasitic on rice plants. *Trans. Tittori. Soc. Agric. Sci.*, **11** : 6482.

MISRA, A. P. AND A. K. MUKHERJEE. (1962) Saltation in *Helminthosporium oryzae* Breda de Haan. *Curr. Sci.*, **31** : 27-28.

RIKER, A. J., AND R. S. RIKER. (1936) *Introduction to research on Plant diseases*. N.Y., U.S.A.

STEVENS, F. L. (1922) The Helminthosporium foot rot of wheat with observations on the morphology of *Helminthosporium* and on the occurrence of saltations in the genus. *Illinois Dept. Registr. and Educ. Div. of Nat. Hist. Survey Bull.*, **14** : 76-185.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE MYXOMYCETES OF CALCUTTA SUBURBS

ASOK KUMAR KAR

(Accepted for publication September 16, 1964)

Investigations on the Myxomycetes of Calcutta have been started with a view to work out in detail the various aspects of the assimilative and reproductive phases of these organisms. Following is a report on some of the Myxomycetes belonging to family Physaraceae that were collected from Calcutta suburbs during early July of 1962.

I. *Craterium minutum* (Leers) Fries

On dead bamboo leaves, Sarsuna (Southern part of greater Calcutta).

Gregariously growing fruit bodies were found along the trail of the plasmodium which developed on the surface of dead bamboo leaves (Fig. 1). The sporangia are stalked, cylindric or goblet-shaped, 0.73-1.25 mm. high arising from a discoid deep-brown coloured hypothallus. The colour of the sporangia varies from yellow to light-yellow. The stalk is light-brown in colour and 0.3-0.5 mm. high with longitudinally running wrinkles. The mouth of each sporangium is covered with a very delicate lid, in some, due to dehiscence by lid the sporangia resemble minute birds' nests. Spores with capillitium of hyaline slender threads are seen to liberate out from the sporangia. Lime granules are present in the knots of the capillitium which combine in the centre of the sporangia forming a pseudo-columella. The colour of the lid is paler than the peridium. The sporangium proper measures 0.43-0.8 mm. in length and 0.29-0.51 mm. in width with outer surface minutely ridged and furrowed alternately. The spores are round, light-brown coloured with warty wall ranging from 7 to 8.5μ in diameter.

The specimens have resemblance with those collected by Miss G. L. Drake from Landour and Darjeeling growing on dead wood and leaves, and described by Lodhi (1934); also with those collected by Agnihothrudu in 1957 growing on undetermined leaves found in decaying vegetable litter from Jorhat, on leaves of *Grevillea robusta* A. Cunn., on leaves of *Mesua ferrea* L, Tyroon, Tea Estate, on an undertemined leaf, Tocklai and were subsequently described by him in 1959.

II. *Leocarpus fragilis* (Dicks.) Rost.

Growing on a dried immature coconut fruit, Sarsuna (Southern part of greater Calcutta).

Ovoid short-stalked sporangia growing on the surface of a dried immature coconut fruit (Fig. 2). The sporangia developed on a rudimentary hypothallus are 1-1.3 mm. in length and 0.5-0.7 mm. wide. The sporangial stalk being very weak the sporangium proper is not held erect resulting in its lying almost on the surface of the substratum. The sporangial wall surface is smooth and shining chestnut brown in colour and is composed of two layers—the outer layer is very thin and brittle and the inner one thick and firm, dehiscence irregular. Pale-brown spores 9-12μ in diameter possess spinulose wall. The spores are clustered along with capillitium in which lime-knots are present. The capillitium is of duplex type comprising of irregularly branched calcareous yellowish orange and of dense network composed of non-calcareous hyaline threads.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

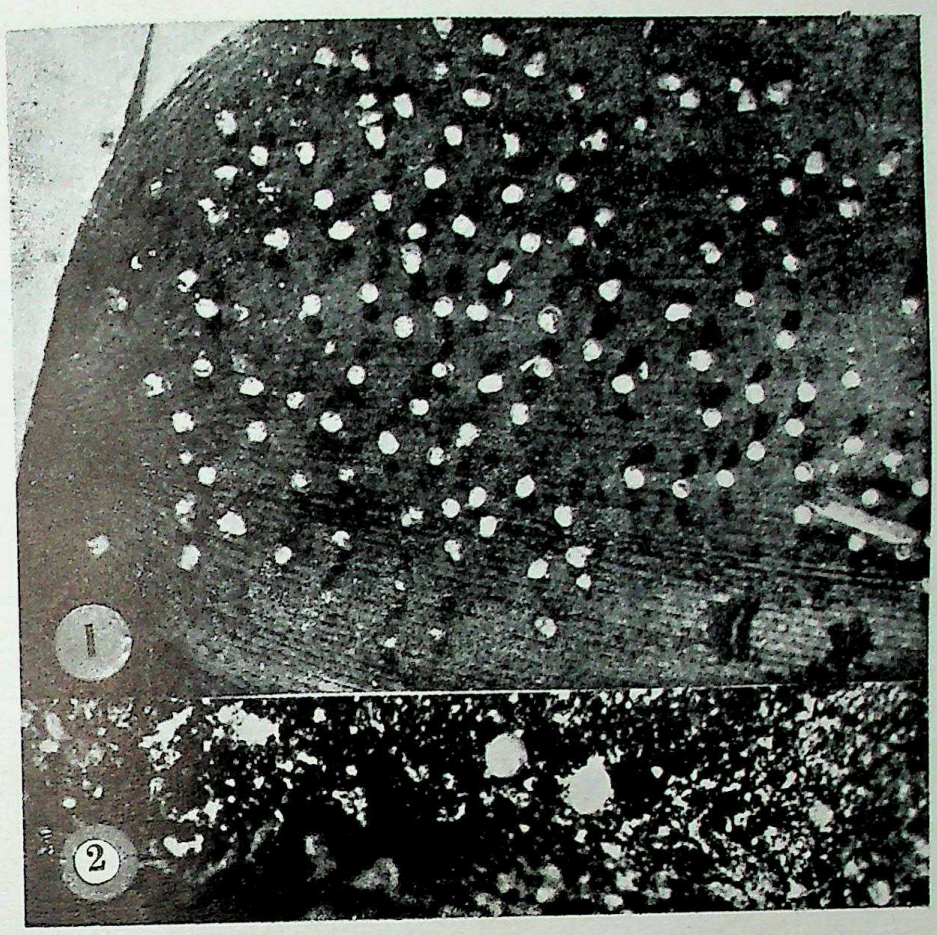


Fig. 1. Sporangia of *Craterium minutum*, ×15.
Fig. 2. Sporangia of *Leocarpus fragilis*; ×20.

The specimens collected and described by Thind and Manocha (1958) from Kantal forest, Mussoorie in August, 1956 growing on bark of *Cedrus deodara* and from Kodia forest in August, 1956 growing on dead coniferous needles (of *Pinus* and *Cedrus*) and on dead wood have similarity with the specimens collected by the author.

Mycology and Plant Pathology Laboratory,
Department of Botany, Presidency College, Calcutta.

References

AGNIHOTHRUDU, V. (1959) Notes on fungi from North-East India-IV. *J. Indian bot. Soc.*, **38** : 453-491.

BUTLER, E. J. AND G. R. BISBY. (1954) *The Fungi of India*, (Revised by R. S. Vasudeva), Indian Counc. Agric. Res., New Delhi.

LISTER, A. (1925) *A Monograph of the Mycetozoa*, (Ed. 3), British Museum, London.

LODHI, S. A. (1934) *Indian Slime-moulds* (*Myxomycetes*). Publ. Univ. Punjab., 1-34.

THIND, K. S. AND M. S. MANOCHA. (1958) The Myxomycetes of the Mussoorie Hills-IX. *Indian Phytopath.*, **11** : 10-22.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ENZYMIC STUDIES OF CERTAIN WOOD DESTROYING FUNGI[1]

D. K. SAHA*

(Accepted for publication August 30, 1964)

INTRODUCTION : The enzymes were recognized as early as 1833 by Payen and Persoz, but actual work in connection with their presence in wood rotting fungi started only in 1895 with the work of Bourquelot and Harissey on *Polyporus sulphureus* and later on by certain other workers (Buller, 1906; Menon, 1934; Bose and Sarkar, 1937; Kenneth, 1938; and Damle, 1952). The present investigations were undertaken with a view to elucidate the enzyme make-up of three wood destroying fungi *Polystictus sanguineous* (Linn.) May. (PS); *Xylaria turgida* Fr. (XT) and *Chaetomium orientum* Saha[2] (CO). Of these *P. sanguineous* has been worked out by certain workers briefly, whereas the enzymes of other two fungi are not worked out. The underlying idea behind the present studies was to find out the enzyme make-up of these fungi. In this paper an account is given of the enzymes detected by means of qualitative procedures.

MATERIAL AND METHODS : Pure cultures of these fungi were utilized for this investigation. Culture filtrates for enzyme determination (extra and intra-cellular) were prepared from young cultures grown for two weeks at a temperature range of 28-30°C in Erlenmeyer flasks containing 25 cc of Richard's solution (Bose and Sarkar, 1937; Ramakrishnan, 1941). The medium was freed of the mycelium and spores by filtering through No 42 Whatman's ashless filter paper in Buchner funnel connected to a suction pump for rapid filtration and stored with a few drops of toluene. The metabolic solution thus obtained served as the extracellular enzyme extract.

In addition to this method, the capacity of the fungus to grow on certain media was also taken as an index of the formation of particular extracellular enzyme (Uppal and Kulkarni, 1937). For this purpose a stock medium of the following composition was prepared - Magnesium sulphate-0.5 gm, Pot. dihydrogen phosphate-1.0 gm, Pot. Chloride-0.5 gm., Ferrous sulphate-trace, Agar-20 gms, and distilled water to make 1000 cc. To this stock medium (SM) different compounds such as Pyragallol, Methylene blue etc. were added and good fungal growth on a particular source was taken as positive test. The media and the solutions containing carbohydrates were sterilized at 8 lbs pressure for 24 mts. for three successive days and the pH was maintained at slightly above 7.

The mycelial mat, washed free of the adhering media, was dried between the folds of sterilized filter paper and then in a desiccator over anhydrous calcium chloride for 10 days, The dried mycelial mat was mixed with a small quantity of acid washed silver sand (20 parts sand to 1 part mycelium) and ground well in distilled water. The mycelial extract was filtered and the filtrate stored with a few drops of toluene. This served as intracellular enzyme extract.

---

[1]Part of the thesis submited for M. Sc. (Ag.) at Banaras Hindu University.

[2]A new species reported by the author.

*Present Address : Mycologist (Production), Antibiotics Project, Virbhadra, Rishikesh, U. P.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RESULTS : The presence of enzymes in the extra and intracellular extracts was tested by procedures referred under each test. The enzyme extract, inactivated by autoclaving at 15 lbs. for 20 mts. was taken as control for comparison. The results are presented in Table 1.

TABLE 1 : Qualitative demonstration of enzymes

Enzymes	Substrate	Incubation time and temperature	Enzyme activity Extracellular PS	CO	XT	Intracellular PS	CO	XT	Method
I. Carbohydrate metabolism									
1. Amylase	Starch (5%)	24 hrs at 37°C	+	+	—	+	—	+	Fehling's solution.
2. Invertase	Sucrose (2%)	—do—	—	—	—	+	—	—	—do—
3. Zymase	Glucose(1%)	—do—	—	—	—	—	—	—	—do—
4. Maltase	Maltose(1%)	—do—	+	—	+	Not done			Barfoed's reagent
5. Protopectinase	Potato discs.	12 hrs at 37°C	—	—	—	+	—	—	Maceration
6. Cellulase	Filter paper	20 days at 37°C	+	+	+	+	+	—	—do=
II. Protein metabolism									
1. Rennatase	Milk (fresh)	45 min at 37°C	+	—	—	+	—	—	Coagulation
2. Protease	Gelatin(7%)	24 hrs. at 37°C	+	+	+	—	—	—	Liquifaction
III. Respiratory enzyme									
1. Oxidase	Pyragallol (0.1%)	7 days at 37°C	+	+	+	Not done			Brown zonation, SM. Bavendaum (1928)
2. Reductase	Methylene blue	—do—	—	+	+	—	—	—	Colour change SM

+ Denotes the presence of enzyme ; — Denotes absence of enzyme

The table shows the presence of a large number of enzyme systems and records for the first time the presence of amylase, protease, oxidase, cellulase, reductase in *C. orientum* and amylase, protease, rennatase, oxidase, reductase, cellulase, and maltase in *X. turgida*.

DISCUSSION : The presence of a large number of enzymes in *P. sanguineous* indicates its wide host range as is the case with other species of this genus (Lefuze, 1937; Kenneth, 1938) and it has been reported on many types of wood. The lesser number of enzymes secreted by *X. turgida* suggests its specificity for a particular substrate. This is substantiated by

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the fact that all the three records of *X. turgida* are on tamarind wood showing its preference for that host. Similarly the enzymes secreted by *C. orientum* are also more or less limited in number.

These qualitative studies showing significant activities of various enzyme systems indicate possibilities for quantitative investigations.

SUMMARY

The enzyme make-up of three wood destroying fungi - *Polystictus sanguineus*, *Xylaria turgida* and *Chaetomium orientum* were studied qualitatively. These were found to produce a number of extra and intracellular enzymes, maximum being in *P. sanguineous* followed by *C. orientum* and *X. turgida*.

ACKNOWLEDGMENT : My thanks are due to Prof. A. Lal and Mr. V. P. Tiwari, College of Agriculture, Banaras Hindu University for their kind guidance and interest in the present work; and to Dr. S. K. Mukherjee, Mycologist West Bengal, for his help in critically going through the manuscript.

Mycology Section,
Rice Research Station,
Chinsurah, W. B.

REFERENCES

BULLER, A. H. R. (1906) The Enzymes of *Polyporus squamosus*. *Ann. Bot., Lond.*, **20** : 49-59.

BOSE, S. R. AND S. N. SARKAR, (1937) Enzymes of wood rotting Polypores. *Proc. roy. Soc.*, **123** : 193-213.

CRABILL. G. H. AND H. S. REED, (1915) Activity of microorganisms with special reference to the production and activity of enzymes. *Bio. Chem. Bull.*, **4** : 30-44.

COLOWIC, N. O. AND S. P. KAPLAN, (1955) *Methods in Enzymology*. Academic Press, New York.

DAMLE, V. P. (1952) Enzymic study of certain parasitic fungi. *J. Indian bot. Soc.*, **31** : 13-55.

KENNETH, H. G. (1938). Studies of *Polyporus abiatinus*. I. The enzyme producing ability of the fungus. *Phytopathology*, **28** : 839-845.

LEFUZE, H. H. (1937) Nutrition characteristics of certain wood destroying fungi. *P. betulinus*, *P. pinicola*, *P. versicolor*. *Plant Physiol.*, **12** : 625-646.

MENON, K. P. V. (1934) Studies in the physiology of parasite, comparison of various enzymic extracts obtained from parasitic fungi *Ann. Bot., Lond.*, **48** : 187-209.

RAMAKRISHNAN, T. S. (1941) Studies in physiology of genus *Colletotrichum*. *Proc. Indian, Acad. Sci. (B.)*, **13** : 60-70.

SAHA D. K. (1964) A New Species of Chaetomium on the Wood of Citrus (*Citrus medica* Linn.) tree. *Sci. and Cult.*, **30** : 351.

UPPAL, B. N, AND N. T. KULKARNI (1937) Studies on Fusarium wilt in Sunnhemp. *Indian J. agric. Sci.*, **7** : 413.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FUNGAL MICROFLORA OF SEEDS OF JOWAR (SORGHUM VULGARE), ITS ROLE IN REDUCED EMERGENCE AND VIGOUR OF SEEDLING AND CONTROL

R. L. MATHUR AND S. P. SEHGAL

(Accepted for publication August 18, 1964)

In India, seed treatment of Jowar with copper carbonate (Uppal and Desai, 1931) and sulphur (Kulkarni, 1944) was found to control the seed-borne grain smut [*Sphacelotheca sorghi* (Link) Clinton]. In recent years, these fungicides have been partly replaced with organomercurial compounds. In addition to controlling externally seed-borne diseases, the seed dressing fungicides are known to have fungicidal action on the seed rotting and damping off fungi, usually carried on seed coat and thereby improve the stand and vigour of seedlings.

After an examination of microflora of 23 lots of sorghum seeds, Leukel and Martin (1943) reported that spores of *Alternaria* sp. predominated, followed by *Fusarium*, *Penicillum Aspergillus*, *Rhizopus* and *Trichoderma* in abundance while *Sphacelotheca sorghi* and *Helminthosporium* occurred occasionally. Bain and Edgerton (1943) pointed out the presence of the fungus *Gloeocercospora sorghi* in seeds of sorghum which was later confirmed by Antonio (1949). Bain (1950) reported *G. sorghi* and unidentified species of *Acrothecium*, *Phoma*, *Fusarium*, *Chaetomium*, *Alterneria*, *Penicillium* and *Hormodendron* on and in the seeds of sorghum. Purss (1953) isolated from the sorghum seeds three species of *Alternaria* and one each of *Aspergillus*, *Rhizopus*, *Heterosporium* *Helminthosprium* and *Fusarium*. Leukel (1943), and Leukel and Webster (1953) reported beneficial effect of fungicidal treatments in increasing the percentage emergence of sorghum seeds.

No work seems to have been carried out in this country on the study of the fungal flora of stored jowar seeds, its role in reducing germination and vigour of seedling and benefit, if any, accruing by seed treatment with fungicides. The present investigations were taken up to study this aspect of the problem and the results are presented in this paper.

MATERIAL AND METHODS : Four varieties of edible Jowar R. S.-1, Kota selected, Ujjain No. 1 and local market seeds were used for the study of the microflora and Kota selected for other experiments. Ten seeds were taken in each replication. Potato-dextrose, Oat meal and Smith Hunifeld agar media were used for plating of seeds. Sterilization of seeds was carried out by 1½ minute dip in 0.1 per cent mercuric chloride solution followed by washing in sterilized water and drying between sterilized blotting papers. For contamination, the sterilized seeds were shaken with 4 weeks' old culture multiplied on potato dextrose agar slants. There were [illegible] plates for each treatment and the experiment was repeated

T'thri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Number of colonies expressed in percentage developing in different media from seeds of four varieties of Sorghum after 72 hours

Medium		Potato-Dextrose-Agar							Oat agar							Smith Humfeld						
Variety		Alt.	Asp.	Rhi.	Muc.	Peni.	Bact.	Mis.	Alt.	Asp.	Rhi.	Muc.	Peni.	Bact.	Mis.	Alt.	Asp.	Rhi.	Muc.	Peni.	Bact.	Mis.
1. R. S. 1	Unsterilised	20	20	27	2	8	10	13	20	11	21	2	2	27	16	20	17	27	10	2	7	17
	Sterilised	3	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2. Kota selected	Unsterilised	40	15	20	2	8	15	—	34	11	26	6	9	14	—	36	14	29	—	3	18	—
	Sterilised	3	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
3. Ujjain No. 1	Unsterilised	9	57	11	6	8	9	—	—	72	12	4	—	12	—	6	53	18	2	2	19	—
	Sterilised	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	3	1	—	—	—	—
4. Kota market	Unsterilised	19	25	42	2	4	6	2	23	17	25	—	8	17	5	19	28	30	—	2	17	4
	Sterilised	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Alt.=Alternaria ; Asp.=Aspergillus ; Rhi.=Rhizopus ; Muc.=Mucor ; Peni.=Penicillium ; Bact.=Bacteria ; Mis.=Miscellaneous.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

For studying the actual role played by the different fungi on the emergence and vigour, sterilized seeds and those contaminated with different fungi separately and collectively were grown in 6" pots containing sterilized soil. After 10 days of sowing, observations were recorded on the number of seeds germinating, height of seedlings after careful uprooting, washing and removing the excess water with blotting paper.

For the study of the effect of the fungicides on different fungi, unsterilized seeds, sterilized seeds as such, and those contaminated with the three fungi separately and collectively were treated with two usually recommended doses of each of the eight readily available fungicides namely, Agrosan GN (Tolylmercury acetate), Harvasan (Organically combined mercury) Ceresan (Ethylmercury chloride), Tillex (Ethylmercury chloride), Lunasan (1-ethylmercuri-2thiourea), Fernasan (tetra methyl thiuram di-sulphide), copper carbonate and sulphur dust by shaking them vigourously for 10 minutes in flasks by hand. Seeds from each lot were then plated on four potato-dextrose-agar plates and the number of colonies developing from the seeds counted after 72 hours. Seeds were also sown in 6" pots and observations on germination, height and weight recorded within 15 days. Four pots were used for each treatment and the experiment was repeated twice.

Microflora of Seed : The detailed quantitative and qualitative observations of the colonies developing from 4 varieties of seeds plated on different media after 72 hours are given in Table 1.

TABLE 2 : Effect of fungal flora on germination, height and weight of seedlings of Kota selected variety of sorghum

Treatment	Germination Percentage	Average Weight in CM.	Weight of 100 Seedlings in in GMS.
Contaminated with *Rhizopus*	72	18.01	57.2
Contaminated with *Aspergillus*	85	18.67	64.5
Contaminated with *Alternaria*	80	18.23	63.3
Contaminated with all the three	67	17.59	56.0
Control	92	22.17	71.5

Species of *Alternaria*, *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium* and bacteria were generally present on almost every variety of the seed, although the colonies of the first three fungi were much more in number than others. It was also observed that there was difference in the percentage of different fungal colonies developing from different varieties, *Alternaria* being predominant on Kota selected, *Aspergillus* on Ujjain [illegible] and *Rhizopus* on market and RS1 varieties. The sediment of centrifuged seed washing, when examined microscopically revealed

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 3 : Germination percentage and number of colonies developing from contaminated and treated seeds

Contaminating fungus	Alternaria			Aspergillus			Rhizopus			All the three					$HgCl_2$ treated seeds (Sterilised)			Non-sterilised				
Fungicides	G. P.	Alt.	Mis.	C. P.	Asp.	Mis.	G. P.	Rhi.	Mis.	G. P.	Alt.	Asp.	Rhi.	Mis.	C. P.	Fungal	Colonies	G. P.	Alt.	Asp.	Rhi.	Mis.
1. Agrosan G. N. 3 ppt.	93	—	—	90	—	—	95	—	—	90	—	—	—	—	100	—	85	85	—	—	—	—
2. ,, ,, 5 ppt.	90	—	—	85	—	—	95	—	—	90	—	—	—	—	90	—		80	—	—	—	—
3. Ceresan 2 ppt.	95	—	—	90	1	—	90	—	—	95	—	1	—	—	85	—		100	—	—	1	—
4. ,, 3 ppt.	100	—	—	90	—	—	97	—	—	90	—	—	—	—	90	—		90	—	—	—	—
5. Harvasan 2 ppt.	97	—	—	90	—	—	90	—	—	95	—	—	—	—	94	—		85	—	—	—	—
6. ,, 3 ppt.	100	—	—	100	—	—	95	—	—	95	—	—	—	—	97	—		95	—	—	—	—
7. Fernasan 3 ppt.	100	—	—	90	1	—	95	3	—	95	—	2	1	—	95	—		100	—	2	—	—
8. ,, 5 ppt.	95	—	—	95	—	—	90	—	—	90	—	1	1	—	90	—		95	—	1	—	1
9. Tillex 2 ppt.	100	—	—	90	—	—	100	—	—	85	—	—	1	—	95	—		90	—	—	—	—
10. ,, 3 ppt.	95	—	—	95	—	—	100	—	—	85	—	—	—	—	100	—		100	—	—	—	—
11. CuCo 4 ppt.	90	1	—	75	9	—	80	5	—	85	—	1	3	—	95	—		85	1	1	3	—
12. ,, 6 ppt.	95	—	—	85	—	—	85	9	—	80	1	—	—	—	100	—		90	—	—	1	—
13. Lunasan 2 ppt.	100	—	—	85	—	—	90	—	—	90	1	—	1	—	80	—		90	—	1	1	—
14. ,, 3 ppt.	90	—	—	95	—	—	90	—	—	95	—	—	1	—	93	—		85	—	—	1	—
15. Sulphur 4 ppt.	95	1	—	90	2	—	95	—	—	80	1	2	3	—	100	—		95	—	1	2	—
16. ,, 6 ppt.	95	2	—	90	—	—	80	3	—	75	1	4	6	—	95	—		95	—	1	3	—
17. Control	90	5	—	85	9	—	75	1	—	65	4	5	11	3	100	—		80	5	5	9	13

PPT.=Parts per thousand. G. P.=Germination percentage. Asp.=Aspergillus. Alt.=Alternaria. Rhi.=Rhizopus. Mis.=Miscellaneous.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 4 : Effect of different fungicides on the germination and vigour of seedlings

Contaminating fungus		Alternaria			Aspergillus			Rhizopus			All the three			H_gC_{12} treated (Sterilised)			Non-Sterilised seed		
Treatment		G. P.	Ht.	Wt.	G. P.	Ht.	Wt.	G. P.	Ht.	Wt.	G. P.	Ht.	Wt.	G. P.	Ht.	Wt.	G. P.	Ht.	Wt.
1. Agrosan G.N.	3 ppt.	84	23.0	41.3	88	22.2	42.2	90	25.3	51.0	85	21.9	40.4	90	23.9	49.6	90	21.9	47.5
,, ,,	5 ,,	90	22.9	52.4	88	21.2	50.8	92	24.5	47.7	87	20.2	45.5	91	26.5	49.9	93	25.9	50.2
2. Ceresan	2 ppt.	85	25.1	41.9	84	22.2	43.7	90	24.0	49.0	82	20.5	40.0	94	21.8	50.0	96	22.4	47.5
,,	3 ,,	87	23.4	36.1	86	23.6	47.5	90	21.7	40.0	83	21.6	43.4	96	22.4	49.9	92	24.4	53.8
3. Harvasan	2 ppt.	55	22.6	90.3	88	21.8	48.3	90	22.8	44.7	88	21.4	50.2	94	24.0	50.3	92	25.4	55.6
,,	3 ,,	93	24.3	59.4	90	25.1	48.9	83	23.8	47.2	85	21.6	52.9	96	22.9	60.3	95	25.9	98.6
4. Fernasan	3 ppt.	88	24.4	56.6	82	25.2	48.9	83	23.8	41.2	85	26.6	52.9	96	22.9	46.3	90	25.9	58.6
,,	5 ,,	93	55.9	22.2	81	21.0	40.1	86	23.6	41.7	80	21.9	47.7	90	25.1	48.0	84	26.0	55.0
5. Tillex	2 ppt.	83	24.5	50.0	80	23.0	50.1	90	23.5	46.1	88	25.2	53.3	90	23.8	55.4	93	25.0	47.4
,,	3 ,,	84	22.8	44.1	87	23.5	53	80	20.3	46.0	85	22.8	46.2	95	24.2	50.0	93	25.1	55.0
6. $CuCo_3$	4 ppt.	88	23.9	46.2	55	22.5	43.7	80	21.6	41.9	88	21.7	42.1	94	26.7	52.9	86	21.8	44.5
,,	6 ,,	94	25.1	49.1	88	20.8	44.2	82	20.3	41.3	86	24.0	47.0	98	26.3	54.1	93	21.5	43.0
7. Lunasan	2 ppt.	87	23.2	44.7	85	22.9	48.5	80	26.6	52.2	80	21.5	49.5	95	25.8	56.8	92	24.9	51.0
,,	3 ,,	87	23.3	43.2	85	23.0	48.1	82	23.6	47.0	82	23.8	48.3	98	25.2	48.7	92	25.2	47.8
8. Sulphur	4 ppt.	83	25.3	45.4	87	23.5	42.1	78	22.9	40.8	78	22.4	45.4	95	22.3	54.4	87	24.5	4[illegible]9
,,	6 ,,	83	26.8	44.0	82	21.2	41.4	82	22.0	43.9	80	20.3	45.5	98	22.5	58.1	88	23.0	45.6
9. Control	(No fungicide)	80	22.6	39.6	82	21.3	40.2	72	22.8	38.2	72	22.3	36.3	98	25.9	53.7	90	21.1	48.0

G. P.=Germination percentage; Ht.=Height of the seedlings in Cms. Wt.=Weight of 100 seedling in gms.
ppt.=parts per thousand.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the presence of spores of *Alternaria*, *Fusarium*, *Curvularia*, *Helminthosporium*, sporangial heads of *Rhizopus*, *Aspergillus* and *Penicillium*; besides oospores and mycelial bits of some unidentified fungi. The most common and predominant fungi isolated were species of *Rhizopus*, *Aspergillus* and *Alternaria* and further work was carried out with them only.

Role of Fungi in Reducing Emergence and Vigour of Seedlings: Seeds contaminated separately and collectively with the three dominant fungi when grown in sterilized soil in 6" pots revealed that the germination percentage was lowest (67 percent) when the seeds were contaminated with a mixture and 72, 80 and 85 respectively with *Rhizopus*, *Alternaria* and *Aspergillus* individually as compared to 92 percent in control. The effect on the height and weight of the seedlings was also in the same order (Table 2).

Efficacy of fungicidal treatment: The observations recorded on the germination and the number of different fungal colonies developing in potato-dextrose agar plates from seeds treated with fungicides are summarised in Table 3. It is evident that fungicides could control the development of fungi, the efficacy of different fungicides differing with different fungi. Although the most effective fungicide was Harvasan, Agrosan GN, Tillex, and Cerersan were also effective. The other fungicides could not check the development of all the fungi at a time. Copper carbonate (4 ppt. and 6 ppt.), Sulphur (6 ppt.) and Fernasan (3 ppt.) proved ineffective for *Rhizopus*. Similarly, Lunasan, Copper carbonate and Sulphur could not fully control the development of *Rhizopus*, *Aspergillus* and *Alternaria*. The fungicidal treatment also resulted in better germination of seeds in each plate.

From the results of pot experiments presented in Table 4, it is clear that fungicides like Agrosan GN, Ceresan, Harvasan and Tillex not only controlled the development of fungi in plates but also induced some stimulating effect and thereby increased the vigour of the seedlings as evidenced by increase in average height and weight of seedlings. Fernasan and Lunasan proved useful in low concentrations only with better germination and vigour and there was retarding effect in higher concentrations. The other fungicides had also some beneficial effect as compared to control but did not show clear stimulating effect on the seedlings. The fungicides were not only effective in checking the pre-emergence loss of seedlings but had definite stimulating effect evidenced by improvement in germination and vigour of seedlings.

SUMMARY

Microflora of four varieties of Jowar seeds was studied. The study made by plating and examining the sediment of the centrifuged seed washings revealed the presence of fungi like *Rhizopus*, *Alternaria*, *Mucor*, *Fusarium*, *Penicillium* and bacteria. The predominant fungi, however, were found to be *Rhizopus*, *Aspergillus* and *Alternaria* in culture. It was observed that the microflora varied with the variety of seed.

The retarding effect of externally seed borne fungi individually and collectively on germination and growth of seedlings was established and the efficacy of seed dressing fungicides determined for check[illegible] the[illegible]

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

losses. The efficacy of fungicides tested was in this desending order, Harvasan, Agrosan GN, Ceresan, Tillex, Fernasan, Lunasan, Copper carbonate and Sulphur.

Seed treatment with fungicides resulted in kill of externally seed-borne fungi and thereby giving better germination percentage, height and weight of seedlings.

The authors are grateful to Dr. N. Prasad, Plant Pathologist, Rajasthan, for critically going through the manuscript and Shri Samrath Raj, Director of Agriculture, Rajasthan, for facilities.

Plant Pathology Section,
Department of Agriculture,
Rajasthan, Jaipur.

References

BAIN, D. C. (1950) Fungi recovered from seed of *Sorghum vulgare* Pers. *Phytopathology*, **40** : 521-522.

———— AND D. W. EDGERTON (1943) The zonate leaf spot, a new disease of Sorghum. *Phytopathology*, **33** : 220-226.

CICCARENE, ANTONIO (1949) Zonate leaf spot of Sorghum in Venezuela. *Phytopathology*, **39** : 760-761.

KULKARNI, G. S. (1944) Baluchistan sulphur for Jowar smut. *Curr. Sci.*, **13** : 48.

LEUKEL, R. W. (1943) Chemical seed treatment for the control of certain diseases of Sorghum. *Tech. Bull. U.S. Dept. Agric.*, 849 : 24.

———— AND J. H. MARTIN (1943) Seed rot and seedling blight of Sorghum. *Tech. Bull. U.S. Dept. Agric.*, 839 : 26.

———— AND O. J. WEBSTER. (1953) Sorghum seed treatment tests in 1935. *Plant Dis. Reptr.*, **37** : 585-586.

PURSS, G. S. (1953) Mould growth on Sorghum seed. *Qd. Agric. J. Sci.*, **10** : 125-126.

UPPAL. B. N. AND M. K. DESAI, (1931) The effectiveness of dust fungicides in controlling grain smut. *Agric. and Livestock in India*. **1** : 396-413.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PRESENCE OF AMINO ACIDS AND INDOLE COMPOUNDS IN THE ROOT-KNOT AFFECTED PLANTS

G. RANGASWAMI AND M. BALASUBRAMANIAN

(Accepted for publication August 26, 1964)

Balasubramanian and Rangaswami (1962) reported on the occurrence of indole acetic acid in the galled roots of bhendi (*Abelmoschus esculentus* Moench) affected by *Meloidogyne javanica* (Treub.) Chitwood. Other workers have reported on the presence of certain amino acids in higher concentrations in the root-knot affected host tissues (Owens and Novotny 1960; Hanks and Feldman, 1963; Krusberg, 1961). With a view to find out whether in the galled extracts of *A. esculentus* different amino acids are present and whether tryptophan and other precursors of indole acetic acid are formed, detailed studies were made and the results are reported here.

MATERIAL AND METHODS : *A. esculentus* seeds were grown in sterilized soil in 6 inch pots. Fifteen days after sowing the soil was inoculated with a suspension of the second stage larvae of the nematode, as per the details given in the earlier reports from this laboratory (Rangaswami *et al.*, 1960). Plants were pulled out at periodical intervals and the free amino acids present in the roots examined, following the procedure of Demetriades (1956). The presence of indole compounds in the plant roots was examined chromatographically, following the procedure reported earlier (Balasubramanian and Rangaswami, 1962).

EXPERIMENTAL RESULTS : (*a*) *Presence of Free Amino Acids* : When the galled as well as healthy roots of *A. esculentus* were examined chromatographically on the tenth day, certain differences in the qualities of the amino acids were found (Table 1) : tryptophan was present only in the galled-root tissues grown in soil and sand cultures and was absent in the non-inoculated plants; histidine was found in the galled-roots raised in soil culture and in the roots of non-infected plants in sand culture; phenylalanine and tyrosine were present only in the galled root tissues of plants raised in sand culture. Twenty days after inoculation the differences observed were : tryptophan was found only in the galled-roots of inoculated plants grown in both sand and soil cultures; glycine and serine were present in the roots of non-infected plants grown in soil culture; arginine was found in the galled-root tissues of soil culture and healthy tissues of sand culture. Thirty days after inoculation, there were some differences in the presence of amino acids, the chief being that tryptophan was not present in the healthy as well as infected roots.

These results indicate that tryptophan, along with some other free amino acids, persist for about 20 days after inoculation. A further comparison of the free amino acids present in the roots of *A. esculentus* in soil and sand cultures, with and without nematode infections, showed that in soil culture the plant root contained serine and glycine whereas

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Free amino acids in the roots of healthy and galled roots of *A. esculentus*

	Plants grown in soil		Plants grown in sand culture	
	Control	Inoculated	Control	Inoculated
10 days after inoculation	Leucine and/or isoleucine, valine, alanine, threonine, glutamic acid and aspartic acid	Leucine and/or isoleucine, valine, tryptophan, alanine, threonine, glutamic acid, aspartic acid and histidine	Leucine and/or isoleucine, valine, alanine, threonine, glutamic acid, aspartic acid, histidine, lysine and cystine	Leucine and/or isoleucine, Phenylalanine, valine, tryptophan, tyrosine, alanine, threonine, glutamic acid, aspartic acid, arginine and cystine
20 days after inoculation	Leucine and/or isoleucine, phenylalanine, valine, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, histidine, lysine and cystine	Leucine and/or isoleucine, phenylalanine, valine, tryptophan, tyrosine, alanine, threonine, glutamic acid, aspartic acid, histidine, arginine and cystine	Leucine and/or isoleucine, phenylalanine, valine, tyrosine, alanine, threonine, glutamic acid, aspartic acid, histidine, arginine, asparagine and cystine	Leucine and/or isoleucine, phenylalanine, valine tryptophan, tyrosine, alanine, threonine, glutamic acid, histidine, lysine and cystine
30 days after inoculation	Leucine and/or isoleucine, phenylalanine, valine, tyrosine, threonine, aspartic acid, histidine, lysine and cystine	Leucine and/or isoleucine, valine, tyrosine, alanine, threonine, aspartic acid, histidine, aspargine, lysine and cystine	Leucine and/or isoleucine phenylalanine, valine, alanine, threonine, glutamic acid, aspartic acid, histidine, arginine and cystine	Leucine and/or isoleucine, phenylalanine, valine, tyrosine, alanine, threonine, glutamic acid, aspartic acid, histidine, arginine and cystine

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

methionine was detected only in the roots grown in sand culture. Also, in these studies there were indications for variations in the concentrations of different amino acids produced under four different sets of conditions, as indicated by the intensity of spots developed with ninhydrin on the chromatograms.

(b) *Presence of indole compounds* : The galled-root extracts from *Nicotiana tabacum* L. and *Solanum melongena* L. gave light blue spots, indicating the presence of indole compounds, whereas no spots developed from the healthy root extracts. The Rf values of the spots from the galled-root extracts of *N. tabacum* and *S. melongena* were 0.53 and 0.14, respectively, as compared to 0.25 for indole acetic acid and 0.44 for indole butyric acid. The spots were identified as hydroxytryptamine and indole-pyruvic acid, respectively (Stowe and Thimann, 1954).

DISCUSSION : Tryptophan was present in the galled-roots of *A. esculentus* when they were assayed 10 and 20 days after inoculation, but could not be traced 30 days after inoculation. This is in agreement with the findings of Krusberg (1961) who isolated tryptophan from the galled tissues of alfalfa caused by *Ditylenchus dipsaci* (Kuhn) Filipjev. Tryptophan in galled-roots might be present as a breakdown product of plant proteins or protein-indole acetic acid complex and more stable if it results from alkaline hydrolysis (Frear 1950, Krusberg, 1961). Perhaps the nematodes set into action the alkaline hydrolysis of proteins in the plant roots. It is also probable that nematodes inject tryptophan as such into plant roots. Indole acetic acid was detected in the extracts of 45 day old galled-roots of *A. esculentus* (Balasubramanian and Rangaswami, 1962). Perhaps the tryptophan formed in the early stages of gall production is utilized for the formation of indole acetic acid, since tryptophan is an immediate precursor of indole acetic acid (Boner, 1950; Mountain, 1960). The difference in number of free amino acids detected in the galls of different ages might be due to the utilization or release of amino acids by the nematode for its own development or conversion into some other compounds including tryptophan (Mountain, 1960). The colour developed in the chromatograms of galled-root tissues was usually denser indicating the presence of amino acids in large quantities as compared to the control. This supports the results of Owens and Novotny (1960) and Hanks and Feldman (1963).

The galled extracts of tobacco and brinjal exhibited hydroxy tryptamine and indolepyruvic acid, respectively, which are believed to be compounds arising from tryptophan and hence precursors of indole acetic acid (Gordan, 1956).

SUMMARY

The presence of various free amino acids in galled and healthy roots of bhendi was examined chromatographically. Presence of tryptophan, an immediate precursor of indole, in the extracts of galled-roots of bhendi grown in soil and sand cultures on 10th day and 20th day, but not on 30th day, after inoculation, was indicated in the chromatogram. The type of free amino acids present in the plants grown in soil and sand

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

cultures differed to some extent. Hydroxytryptamine and indole pyruvic acid were also detected in the galled-root extracts of tobacco and bhendi, respectively.

Microbiology Laboratory,
Department of Agriculture,
Annamalai University,
Annamalainagar, Madras State.

REFERENCES

BALASUBRAMANIAN, M. AND G. RANGASWAMI (1962) Presence of indole compound in nematode galls. *Nature Lond.*, **194** : 774-775.

BONNER, J. (1950) *Plant Biochemistry*, Academic Press Inc., New York, U. S. A.

DEMETRIADES, S. D. (1956) Chromatographic detection of free amino acids in normal and iron defecient plants of *Hibiscus esculentus* L, *Nature Lond.*, **177** : 95.

FREAR, E. H. (1950) *Agricultural Chemistry* De Van Nostrand Company. Inc., New York, U. S. A.

GORDAN, G. A. (1956) The biogenesis of natural auxins. In *The Chemistry and mode of action of plant growth substances*. pp : 65-75. (Ed.) Wain, R. L. and F. Wightman. Butterworths, London.

HANKS, R. W. AND A. W. FELDMAN (1963) Comparison of free amino acids and amides in the roots of healthy and *Radopholus similis* infected grape fruit seedlings. *Phytopathology*, **53** : 419-422.

KRUSBERG, L. R. (1961) Studies on the culturing and parasitism of plant parasitic nematodes in particular *Ditylenchus dipsaci* (Kuhn) Filipjev and *Aphelenchoides ritzemabosi* Steiner, on alfalfa tissues. *Nematologica*, **6** : 181-200.

MOUNTAIN, W. B. (1960) Mechanism involved in plant nematode relationship. In *Nematology*, pp. 426-431. (Ed.) Sasser, S. N. and N. R. Jenkins Univ. N. Carolina Press, Chapel Hill, U. S. A.

OWENS, R. G. and H. W. NOVOTNY (1960) Physiological and biochemical studies on nematode galls. Abst. *Phytopathology*, **50** : 650.

RANGASWAMI, G., V., N. VASANTHRAJAN AND R. VENKATESAN (1960) The occurrence of root-knot nematodes on sugarcane and on some weeds. *Curr. Sci.*, **29** : 236-237.

STOWE, B. AND K. V. THIMANN (1954) The paper chromatography of indole compounds and some indole containing auxins of plant tissues. *Arch. Biochem. and Biophys.*, **51** : 501-516.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PRELIMINARY SCREENING OF WHEAT VARIETIES AGAINST LOOSE SMUT BY THE 'EMBRYO TEST'

R. L. MUNJAL AND M. S. CHATRATH

(Accepted for publication September 28, 1964)

Loose smut of wheat caused by *Ustilago nuda* f. *tritici*, occurs throughout India, wherever this crop is grown and causes considerable losses to this valuable staple crop. Growing of resistant varieties is one of the easiest and cheapest method of disease control. In loose smut the infection is internally seed-borne within the embryo, which expresses itself in the field in the form of smutted ears, when the crop is raised from the infected seed.

Simmonds (1946) described a method of separating wheat and barley embryos from the other parts of the grains. These embryos were then examined for the presence of mycelium of *Ustilago nuda* f. *tritici*. Russell (1950) and Russell and Popp (1951) employed this test for forecasting the severity of loose smut infection in barley. Later, Popp (1958) and Morton (1961) published modified techniques for similar 'Embryo tests'.

This note records the observations made on the embryos of 20 wheat varieties for detecting loose smut mycelium and on the incidence of loose smut in the field raised from the same seed.

MATERIAL AND METHODS : Ten ears each of twenty wheat varieties were inoculated with spore suspension of a mixture of ten isolates of *U. nuda* f. *tritici* at the mid-anthesis stage by Moore's (1936) partial vacuum method. These were covered with butter paper envelopes and harvested when the grains matured. This seed was later employed for embryo test as also sown in the field to record the incidence of smut. Technique employed was the same as suggested by Morton (1961) with slight modification. The Embryo test consisted in soaking the dried grains in 5 per cent solution of sodium hydroxide containing 0.05 percent cotton blue for 15 hours at room temperature. By this treatment, the embryos separate themselves from the grains and at the same time the smut mycelium in the embryos gets stained. These were collected and heated in lactophenol (1 : 1 : 1 : 5 parts of lactic acid, glycerine, phenol and distilled water, respectively) for 4-5 minutes for softening and destaining suitably. Embryos were then allowed to cool, washed in distilled water, crushed and examined under microscope for detecting loose smut mycelium, which is stained blue. Ten to fifteen embryos were examined for each variety.

EXPERIMENTAL AND RESULTS : Infection observed in twenty wheat varieties both in the embryo test and in the field is recorded in Table 1.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Incidence of loose smut in wheat varieties by embryo and field tests

Wheat Varieties	Percentage infection in Embryo Test	Field Test
1. HD 59-505	75.0	6.2
2. HD 59-509 A	70.0	41.5
3. HD 59-509 B	90.0	22.8
4. HD 59-514	40.0	50.0
5. HD 59-517	80.0	48.3
6. HD 60-560	33.0	57.8
7. HD 60-562	40.0	48.0
8. HD 60-563	0.0	44.4
9. HD 60-576	75.0	70.5
10. HD 60-580	62.5	70.0
11. HD 60-1256	0.0	0.0
12. HD 60-1268	0.0	0.0
13. HD 60-1272	0.0	0.0
14. HD 60-1303	0.0	0.0
15. HD 60-1304	0.0	0.0
16. E 3163	0.0	0.0
17. E 671	0.0	0.0
18. H-389-7-76-1-4	11.0	10.0
19. NP 775 mutant V	0.0	0.0
20. NP 775	33.3	30.1

The data in the above table clearly show that out of 20 varieties tested, 13 show similar trend in both embryo and field tests, while six show some difference which may be attributed to the effect of environment on development of the fungus within the host and expression of symptoms. The only exception is HD 60-563, which did not show any mycelium in the embryo test but showed 44.4 per cent smut infection in the field.

DISCUSSION : Strictly speaking, the presence of intraseminal mycelium in wheat embryo does not necessarily mean that it belongs to the loose smut fungus, *U. nuda* f. *tritici*, but for all practical purposes it means so, because at the moment, we know of no other fungus whose mycelium remains in dormant state within the seed. It is further confirmed in the observations reported above that of the eleven varieties that showed intraseminal mycelium, all developed smutted ears. This indicates that embryo test can be safely and usefully employed for quick screening of a large number of wheat varieties or for certification of wheat seed for loose smut.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The only exception is HD 60-563 in which intraseminal mycelium could not be detected but developed 44.4 per cent infection in the field. In the embryo the mycelium is present only in small bits and there are chances that with the present technique it may not be located in some cases. This shows that the method though quite useful for quick preliminary screening of varieties, is not infallible.

In cases where embryo does not show mycelium, field tests should be conducted to confirm the reaction of the varieties.

ACKNOWLEDGEMENTS : Sincere thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for encouragement, valuable guidance and keen interest in this work. Thanks are also due to Shri Chanan Singh for assistance in the routine work.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

MOORE, M. B. (1936) A partial vaccum method for the inoculation of wheat and barley with loose smut. *Phytopathology*, **26** : 103.

MORTON, DONALD, J. (1961) Trypan blue and boiling lactophenol for staining and clearing barley tissues infected with *Ustilago nuda*. *Phytopathology*, **51** : 27-29.

POPP, W. (1958) An improved method of detecting loose smut mycelium in whole embryo of wheat and barley. *Phytopathology*, **48** : 641-643.

RUSSELL, R. C. (1950) The whole embryo method of testing barley for loose smut as routine test. *Sci. Agric.*, **30** : 361-366.

RUSSELL, R. C. AND W. POPP (1951) The embryo test as a method of forecasting loose smut infection in barley. *Sci. Agric.*, **31** : 559-565.

SIMMONDS, P. M. (1946) Detection of the loose smut fungi in embryos of barley and wheat. *Sci. Agric.*, **26** : 51-58.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PERITHECIAL PRODUCTION BY DIFFERENT STRAINS OF GLOMERELLA TUCUMANENSIS (SPEG.) ARX AND MUELLER

B. L. CHONA, B. S. BAJAJ* AND RAJESHWARI SHARMA

(Accepted for publication August 30, 1964)

It has been repeatedly observed that certain isolates of *Colletotrichum falcatum* Went [*Glomerella tucumanensis* (Speg.) Arx and Mueller], the causal organism of sugarcane red rot, produce perithecia readily and abundantly on old dry autoclaved leaves of sugarcane whereas certain other strains either do not produce the sexual stage or produce scantily (Chona and Srivastava, 1952 ; Chona, Bajaj, Agrawal and Sreenivasan, 1960). Such a differential behaviour of different isolates could be due to either natural strain differences or the loss of ability to produce perithecia during their maintenance on artificial culture media. The studies conducted in this respect are reported here.

Large-scale isolations were made both from leaf midrib lesions and diseased stalks of sugarcane of 64 varieties collected from 46 different localities of Punjab, Delhi, Himachal Pradesh, Uttar Pradesh, Bihar, West Bengal, Orissa, and Kerala States on oatmeal agar and cultures were purified by single-spore isolations. One hundred and ninety-two isolates were tested for their ability to produce perithecia by the usual method (Carvajal and Edgerton, 1944), using dry old leaves of Co.647. Care was taken to select leaf pieces which were free from mould infestation. Uniformly cut leaf pieces were inoculated, after autoclaving, by dipping the entire leaf piece into spore suspension of the isolate concerned and incubated in moist chamber at 25°C. Observations on the perithecial formation were recorded after 10 days and their maturity ensured by examining the crushed perithecia under the microscope. The isolates were categorised into perithecia forming (fertile) and non-perithecia forming (sterile) strains on the basis of their reaction on dry autoclaved leaves of sugarcane variety Co.647. Efforts were made to test the isolates as soon as these were cultured and purified, thus avoiding prolonged subculturing on the artificial medium. The maximum period between isolation and testing was about 4 months which necessitated subculturing only once. The data showing the natural population of perithecia-forming and non forming isolates of *C. falcatum* are given in Table 1.

TABLE 1 : Population of fertile and sterile isolates of *C. falcatum* in the isolations made from different localities and different varieties

Number of isolates tested	Whether isolated from leaf or stalk	Morphological category	No. of isolates	
			Fertile	Sterile
134	Stalk	Light	0	134
13	Stalk	Intermediate	1	12
45	Leaf	Dark	45	0

*Present address :—Deputy Superintendent, Antibiotics project, Rishikesh (U.P.)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It would be observed that all the isolates from leaf were of dark type and produced perithecia on cane variety Co.647 whereas all the stalk isolates were morphologically light or intermediate and did not produce perithecia except only one which was morphologically intermediate between the two and produced mature perithecia abundantly, like the leaf isolates.

Further collections of red rot affected samples of leaf and stalk, especially from the same clump of sugarcane varieties, Co.312 and Co.1281, were made from Delhi State and Punjab and strains purified after isolation. Leaf isolates which resembled dark type produced abundant perithecia whereas the stalk isolates of the same clump, which were light sporulating type were found to be sterile, though these produced abundant conidia. It, therefore, appears that there is strain differentiation between the red rot flora prevalent on leaf and stalk so far as the production of ascigerous stage and morphological characteristics are concerned.

This concept was further confirmed by inoculating two sterile light type stalk-isolates, *viz.*, Cf 550 and 560 on leaf mid-rib and two fertile, dark type leaf-isolates, *viz.*, Cf 515 and 516 on stalk of growing canes of Co.647. The inoculations in the former case were made by spraying the conidial suspension on the surface-sterilised leaf midrib of growing plant with slight injury by means of a fine entomological pin (No. 20) and covering the inoculated portion by means of sterilised moist cotton-wool swab. Inoculated plants were kept in humid chamber for 48 hours and then placed in the open. Typical *C. falcatum* lesions developed on the mid-rib portion. The stalk inoculations were made by the Standard Plug Method (Chona, 1954). Re-isolations were made from the infected portions of leaf and stalk after an incubation of about 3 weeks (March, 1963) and the isolates, after purification, tested for perithecial development in both the cases, keeping the original isolates maintained on oatmeal agar as control. The stalk isolates, in spite of their passage through leaf, failed to produce perithecia ; and the leaf isolates did not lose their capacity to bear perithecia even though these remained growing on the stalks for nearly three weeks. There was no discernible change in the morphological characters of these isolates either as a result of passage through host portions other than the original habitat showing thereby that the leaf and stalk isolates of red rot fungus are distinct in respect of these characters, namely colony colour and perithical production. It is possible that different levels of nutrition available in the two cases (leaf and stalk) may be the contributing causes in screening physiologically distinct strains on the two substrata so that the nutritional requirement of different isolates to perform a particular function (perithecial production in the present case) would vary ; or there may be genetical differences between different types of isolates which may be the cause of this differential behaviour. Keeping this in view, an experiment on perithecial production was set up using old, dry leaves and fully mature green leaves of sugarcane variety Co.647 after surface sterilisation with 95 per cent alcohol and steam sterilisation by autoclaving at 15 lb. for 15 minutes and inoculating with 9 stalk isolates, *viz.*, Cf 244, 471, 479, 492, 498, 500, 502, 503 and 506. The results regarding

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

perithecial production were recorded after 20 days and are given in Table 2.

TABLE 2 : Perithecial production by the stalk isolates on green leaves and old dry leaves of sugarcane

Isolate No.	Perithecial production			
	Green leaves		Old Dry leaves	
	Surface sterilised	Autoclaved	Surface sterilised	Autoclaved
Cf. 244	+	—	—	—
,, 471	+	—	—	+
,, 479	—	—	—	—
,, 492	—	—	—	—
,, 500	—	—	—	—
,, 502	—	—	—	—
,, 503	—	—	—	—
,, 506	—	—	—	—
Control-Leaf isolate Cf. 515	—	—	—	+

As none of the stalk isolates produced peirthecia on old dry leaves except one and on green leaves except two, it appears that the differences between different isolates are genetically controlled and not influenced by the nutrition of the host from which these have been originally isolated. Isolate Cf 471 appears to be genetically distinct as it produces perithecia both on green-surface-sterilised and old dry autoclaved leaves of sugarcane. It is interesting to note that isolate No. Cf. 244, which has been maintained on artificial medium for over 16 years (isolated during 1947 from Co. 312), produced perithecia on green, surface-sterilised leaves and not on any other substrata tried so far.

SUMMARY

Large-scale isolations of red rot pathogen from 64 sugarcane varieties collected from 46 different localities both from stalk and leaf mid-rib showed wide range of morphological variability as also in their ability to produce perithecia. In general, the leaf isolates were dark type and produced perithecia on old dry autoclaved sugarcane leaves whereas the stalk isoates were light type and failed to produce ascigerous stage, indicating strain differentiation on the two substrata. Passage through the host parts other than original habitat of leaf and stalk isolates did not affect any change of the above two characteristics, thus confirming the existence of strain differentiation on leaf and stalk of sugarcane.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Some of the stalk isolates produce perithecia on surface-sterilised green leaves which is an unsuitable substratum for perithecial production so far as leaf isolates are concerned.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute, New Delhi.

References

CARVAJAL, F. AND C. W. EDGERTON (1944) The perfect stage of *Colletotrichum falcatum*. *Phytopathology*, **34** : 206-213.

CHONA, B. L. (1954) Studies on Diseases of Sugarcane in India. IV. Relative resistance of sugarcane varieties to red rot. *Indian J. agric. Sci.*, **24** : 301-316.

CHONA, B. L. AND D. N. SRIVASTAVA, (1952) The perithecial stage of *Colletotrichum falcatum* Went in India. *Indian Phytopath.*, **5** : 158-160.

CHONA, B. L.; B. S. BAJAJ; H. O. AGRAWAL AND T. N. SREENIVASAN, (1960) : A note on the factors influencing perithecial production in *Glomerella tucumanensis* (Speg.) Arx and Mueller. *Proc. IVth All-India Conf. Sugarcane Res. and Develop. workers*, Waltair.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ROLE OF VULPIA MYUROS AND BRIZA MINOR IN THE PERPETUATION OF BLACK RUST OF OATS IN THE NILGIRI HILLS

L. M. JOSHI AND V. C. LELE

(Accepted for publication September 30, 1964)

Vulpia myuros (L.) C.C. Gmel. [=*Festuca myuros* subsp. *pseudo-myuros* (Soy. Will) Hayw.] and *Briza minor* L. are two common grasses in the wastelands and pastures of the Nilgiri hills. Both these were introduced in the South Indian hills as ornamental plants and have escaped to wilderness. They have now become firmly established and naturalised in Nilgiri hills and are commonly found infected with black rust (*Puccinia graminis* (Pers.) in almost all seasons of the year. Black rust has been collected on these grasses during spring, summer, autumn and winter months for several years.

Some earlier workers (Mundkur, 1949, Ramakrishnan *et al.*, 1953) have claimed that *V. myuros* and *B. minor* get infected by *Puccinia graminis* var. *tritici* (Pers.) Eriks and Henn. It has been suggested by them that these grasses help in perpetuation of black rust of wheat from one season to the next by harbouring the infection during summer months, a short gap season between two successive crops, that is, October-April and June-September. Ramakrishnan *et al.*, (l.c.) have also stated that both these grasses also get infected by *Puccinia graminis* var. *avenae* (Pers.) Eriks. and Henn.

Vasudeva, Joshi and Lele (1953) had earlier recorded that both the grasses were highly susceptible to black rust of oats and pointed out the danger of indiscriminate introduction of exotic grasses particularly in the hills.

The data presented in this paper are based on the work mainly carried out between 1948 and 1954. The work was started in 1948 when two samples, one of *V. myuros* and the other of *B. minor* were obtained from the Nilgiri hills. The samples were established on a local variety of Oats (*Avena* sp.) grown in Punjab and on analysis yielded some common races of black rust of oats. Since then samples of rust from these two grasses were obtained year after year from Nilgiri hills. For establishing rust cultures, samples from these hosts were put on wheat, barley, rye and oats and in all cases, without exception, only oats got infected. The cultures were maintained for a number of successive generations on the variety of oats mentioned above and the rust was successfully transferred back on original hosts without difficulty. The other cereals, namely, wheat, barley and rye never took up infection in these tests. In the later stages of this work, whenever a sample from these grasses was received, the rust was put on oats, barley, rye and a number of wheat varieties, such as Agra local wheat (a variety of *T. aestivum* highly susceptible to black rust), Little Club (*T. compactum*), Khapli (*T. dicoccum*), Einkorn (*T. monococcum*), Kubanka and Acme (both *T. durum*). The use of such a wide range of cereal hosts was considered necessary for eliminating any chances of not establishing the infection due to resistance of one particular host. In all these cases, however, rust samples of *V. myuros* and *B. minor*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

never established on any other cereal host or its varieties except oats, whereas the local variety of oats from Punjab always showed a high degree of susceptibility and could be used freely for maintenance of cultures, generation after generation. The first two samples of rust from each of the two grasses under study were maintained on oats at Simla Laboratory during 1948-51.

During 1948-54, a number of samples of rust-infected *V. myuros* and *B. minor* were obtained and established for analytical study. Table 1 gives the identity of physiologic races of *Puccinia graminis* var. *avenae* occurring in them,

TABLE 1 : Physiologic races of black rust of oats identified in samples from *Vulpia myuros* and *Briza minor* from the hilly regions of South India

Year of collection	Locality	Host	Race/s met with
1948	Nilgiri hills*	*V. myuros* (2 samples)	4 and 7
		B. minor (2 samples)	3
1949	Ootacamund	*V. myuros*	4
	Sholada	*V. myuros*	4
	Wellington	*V. myuros*	6
	Ootacamund	*B. minor*	4
	Sholada	*B. minor*	4
	Wellington	*B. minor*	4
1950	Nilgiri hills*	*V. myuros*	3
	Nilgiri hills*	*B. minor*	3 and 7
1951	Ootacamund	*V. myuros*	4 and 6
	Nanjanad	*V. myuros*	6
	Wellington	*V. myuros*	4
	Sholada	*V. myuros*	6
	Ootacamund	*B. minor*	4
	Wellington	*B. minor*	4
	Sholada	*B. minor*	4
	Nilgiri hills*	*B. minor*	4
1952	Nanjanad (2 samples)	*V. myuros*	3, 4 and 6
	Ootacamund	*V. myuros*	3
	Nanjanad (2 samples)	*B. minor*	3
	Ootacamund	*B. minor*	3
1953	Ootacamund (2 samples)	*V. myuros*	3 and 7
1954	Wellington	*V. myuros*	4
	Wellington	*B. minor*	4

*Exact locality of these samples is not known.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

On the basis of cross-inoculation tests, as well as analytical study of rust samples, it is evident that both the grasses get infected only by black rust of oats (*Puccinia graminis* var. *avenae*).

Glasshouse Experiments :

(a) *Black rust of oats and wheat* : Side by side, *V. myuros* and *B. minor* were tested with races of wheat rust under optimum glasshouse conditions. Seedlings were raised in spore-proof glasshouse and inoculated with races of oat rust (3, 4, 6 and 7) and wheat rust (15, 21, 24, 34, 40, 42, 72, 75, 117, 122, 194, 15-C and 42-B) individually using both Punjab local oats and Agra local wheat as controls. Whereas each of the four races of oats rust produced heavy infection of type-4 pustules on both *V. myuros* and *B. minor* none of the wheat rust races could infect any of these grasses under normal conditions (90-100 per cent humidity for 4 hours before and 48 hours after inoculation). Three repetitions consistently gave negative results with the races of wheat rust.

In subsequent series of experiments, conditions of infection, particularly humidity, were purposely altered. Seedlings were kept under high humidity for 72 hours before and 48 hours after inoculations and, by this method, very small resistant type of pustules, surrounded by severe necrotic zone (1-type pustules), were produced on a few leaves of *Vulpia myuros* with races 15, 15-C, 24, 42-B and 194 but even in this case, most of the inoculated leaves remained free from infection and showed no signs of infection. *Briza minor* remained immune even under these conditions.

It may be stated here that Prasada (1948), while testing a number of indigenous grasses from the Simla hills with races of black rust of wheat also came to similar conclusions in regard to *Festuca myuros* (=*Vulpia myuros*).

From the present studies, it is clear that *Briza minor* is immune to black rust of wheat and, therefore, the question of its playing any part in the perpetuation or spread of wheat rust does not arise. *Vulpia myuros* is also highly resistant to black rust of wheat under normal conditions, though under extreme conditions of high humidity, small resistant type of pustules may appear on some leaves. It may be appropriate to stress here that for any grass given to play a part as a collateral host, its high susceptibility is a pre-requisite for building up inoculum necessary to maintain continuitiy of the rust from one season to another, yet infection from resistant type of pustules, developed on wheat under extreme conditions, could not be transferred back to seedlings of this grass, which always failed to get infected even under most favourable conditions. The present findings confirm the earlier view that *Vulpia myuros* is highly resistant to black rust of wheat and does not normally get infected. The resistant type of pustules appearing at times under extreme conditions help niether in building up the inoculum, nor in maintaing the continuity of the rust.

Field Experiments :

Plants of *Vulpia myuros* and *Briza minor* were raised at Delhi in 3′×3″ plots along with a susceptible wheat variety in border rows. Two to three month old plants were inoculated in winter months when conditions for rust development are most favourable, with a

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

mixture of races of black rust of wheat by dusting freshly collected uredospores under conditions of high humidity. This was repeated for two years but the plants of both the grasses never took infection, even though the controls (wheat) in border rows gave good infection. The same experiment when repeated with races of black rust of oats in mixture in another season gave, heavy infection on both these grasses, as well as, on the controls (Punjab local oats).

DISCUSSION: On the basis of the information presented above, it is evident that *Vulpia myuros* is highly resistant and *Briza minor* immune to black rust of wheat under field and glasshouse conditions. On the other hand, both these grasses have been found to be highly susceptible to all the four Indian races of black rust of oats so far met with in the country and primarily from the Nilgiri hills. This is further supported by the fact that these very races were encountered in the analytical study of samples carried over a number of years; and in no case, a race of black rust of wheat was found in the samples. In cross-inoculation experiments, the rust from both the grasses could be transferred only to local varieties of oats and back to original hosts; other common cereals always gave negative results. From these results, it is reasonable to conclude that the natural infection of black rust commonly found on these grasses in the region of the Nilgiri hills is not of *P. graminis* var. *tritici* but of *Puccinia graminis* var. *avenae*. Since these grasses are found infected by black rust of oats even during intervening summer season or rainy season (May-July), they can, in all probablity, play a significant part in carrying over this infection from one oats crop to the next but there is no evidence to support the view of Mundkur (1949) and Ramakrishnan *et al.*, (1953) that they harbour black rust of wheat in the same way and also help in its perpetuation. In fact, the evidence obtained in these studies is contrary to their view.

ACKNOWLEDGEMENT: We wish to record our gratitude to late Dr. K. C. Mehta who advised us to take this problem and suggested the lines of work. Thanks are also due to Dr. R. S. Vasudeva and Dr. R. Prasada for their helpful suggestions during the course of these investigations and to Dr. B. L. Chona for correcting the manuscript. Shri M. H. Rao helped in analyses of some samples and we are thankful to him for his help.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

MUNDKUR, B. B. (1949) *Fungi and Plant Disease*. Macmillan and Co., Ltd., London.

PRASADA, R. (1948) Studies on Rusts of some of the wild grasses occurring in the Neighbourhood of Simla. *Indian J. agric. Sci.*, **18**: 165-176.

RAMAKRISHNAN, T. S., K. V. SRINIVASAN AND N. V. SUNDARAM. (1953) Proceedings of second Scientific Workers' Conference.

VASUDEVA, R. S., L. M. JOSHI AND V. C. LELE (1953) Susceptibility of some grasses to cereal Rusts. *Indian Phytopath.*, **6**: 39-46.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

OBSERVATIONS ON INDIAN AQUATIC FUNGI

II OCCURRENCE OF *OLPIDIOPSIS VARIANS* SHANOR AT GORAKHPUR

G. C. SRIVASTAVA

(Accepted for publication September 16, 1964)

In an earlier communication, Srivastava and Bhargava (1963) have reported on three species of *Olpidiopsis viz.*, *O. saprolegniae* var. *saprolegniae*, *O. fusiformis* and *O. luxurians* from Gorakhpur. Recently the author came across another species of *Olpidiopsis* infecting *Achlya proliferoides* and *Isoachlya anisospora* var. *indica*. Except for a few differences this fungus resembles *O. varians* described by Shanor (1939a) from U.S.A. This fungus has not so far been recorded occurring naturally in these two hosts. In fact, Shanor (1940) was unable to transfer *O. varians* to three species of *Isoachlya viz.*, *I. anisospora*, *I. unispora* and *I. eccentrica*, though it could develop on transfer from *Achlya flagellata* to *A. proliferoides*.

To determine whether the parasite found in the two hosts is similar or different, three attempts were made to transfer it from one host to the other on the lines suggested by Shanor (1939b and 1940). Unifungal cultures of the hosts were prepared on sterilized hemp seed halves. When the hyphae were about 1 mm. long, infected hyphae containing mature sporangia of parasite were removed from the original culture, washed properly in sterilized distilled water and transferred to young cultures of the host. Cross inoculations were successful in all the experiments. Same range of variation as was present in the original infection, was found when the fungus was inoculated in these two hosts.

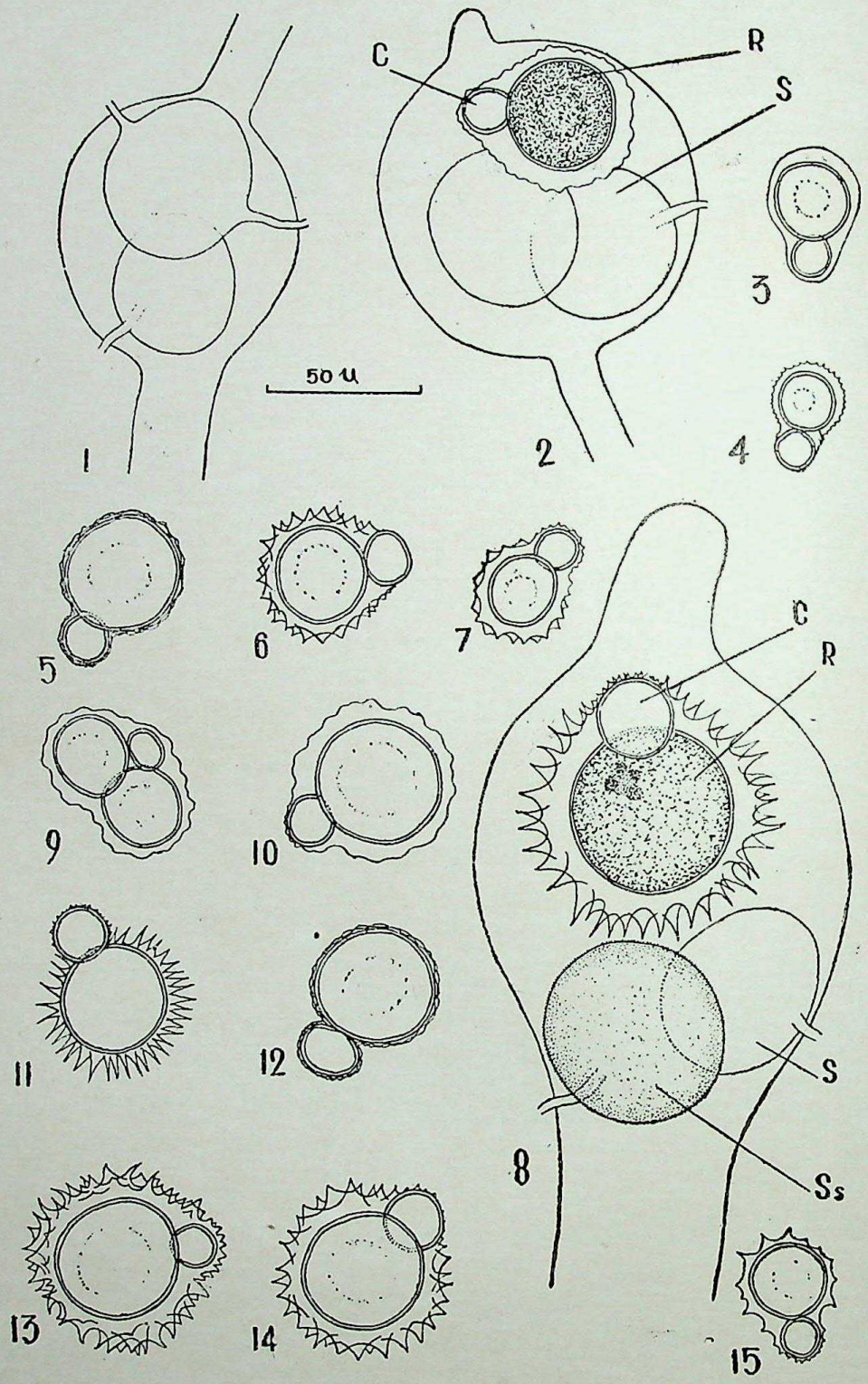
So far only three species of *Olpidiopsis* having spiny companion cells are known. A detailed morphological study of the parasite in the two hosts has shown that this isolate differs from *Olpidiopsis index* Cornu as in the latter the wall of the companion cell is not continuous with that of the resting spore. It also differs from *O. spinosa* Tokunaga which bears very long and rod-like sharp spines on its companion cells. It agrees with *O. varians* described by Shanor (1939a) in the continuation of the thick, generally ornamented exospore of the companion cell with that of the resting spore. There is, however, some variation in the size, spininess and shape of sporangia and also of the resting spore. Shanor (1939a) has also noted the variation in the spininess not only of the sporangia but also of the resting spore. In the present study this variation is apparent not only in the two hosts but in the some host as well. This variability lends further support to Shanor's nomenclature of the parasite.

Olpidiopsis varians :

Thallus endobiotic, holocarpic; *zoosporangia* one to several, usually formed in terminal or occasionally intercalary swellings of the host hyphae, mostly spherical or oval, variable in size, 30-75μ in diameter; wall smooth, colourless; discharge tubes one to

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



For legend see page 250.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

three, narrowly cylindrical, 12-25μ in length; *zoospores* oval to elongated, 3-4.5μ in length, biflagellate; *resting spores* spherical, variable in size, 18.5-45μ in diameter (not including spines), exospore wall colourless or yellowish, varying from 2.5-10.5μ in thickness, with small or large, broad based spines or with an entire, undulant or serrate margin; *companion cells* one or two with each resting spore, usually spherical, 11-22.5μ in diameter, outer wall continuous with that of the resting spore, usually thick and bearing short ornamentations or smooth, colourless or yellowish (Figs. 1-7).

On *Isoachlya anisospora* var. *indica* collected from Gorakhpur.

O. varians :

Thallus endobiotic, holocarpic; *Zoosporangia* one to several in terminal, occasionally intercalary hypertrophied host hyphae, usually spherical or oval, variable in size, 55-100μ in diameter, wall thin, colourless, mostly smooth, sometimes bearing very fine echinulations; discharge tubes one to five, 15-37.5μ in length; *zoospores* oval to elongated, 3-5μ in length, biflagellate; *resting spores* spherical, variable in size, 22.5-60μ in diameter (not including spines), exospore wall colourless to yellowish, 3-18μ in thickness, bearing protuberances, small or large, narrow or broad-based spines, or with an entire undulant or slightly serrate margin; *companion cells* one or two with each resting spore (rarely only one between two resting spores), spherical or oval, 15-27μ in diameter, outer wall continuous with that of resting spore, occasionally smooth but mostly covered by ornamentations similar to those on the resting spore though smaller (Figs. 8-15).

On *Achlya proliferoides* collected from Asuran-ka-Pokhra, Gorakhpur December 7, 1963.

Figs. 1-7. *O. varians* in *I. anisospora* var *indica*.

Flg. 1. Showing two intercalary sporangia with their discharge tubes.

Fig. 2. Showing two sporangia (S) a resting spore (R) with one companion cell (C).

Fig. 3. Resting spore and companion cell surrounded by a smooth exospore.

Fig. 4. Resting spore with companion cell surrounded by serrated exospore.

Fig. 5. Resting spore with companion cell bearing thin warty exospore.

Figs. 6 and 7. Resting spore and companoin cell bearing broad based spines.

Figs. 8-15. *O. varians* in *A. proliferoides*.

Fig. 8. Showing one smooth walled sporangium (S), one sporangium bearing fine echinulations on its wall (Ss) and a resting spore (R) with its companion cell (C).

Fig. 9. A companion cell attached to two resting spores, all surrounded by a common exospore with indulant margin.

Fig. 10. Resting spore with companion cell bearing undulant exospore.

Fig. 11. Resting spore and companion cell bearing long tapering spines.

Fig. 12. Resting spore with companion cell with thin warty exospore.

Figs. 13, 14 and 15. Resting spores with companion cells bearing broad based spines of different shapes.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

DISCUSSION : The present isolate of *Olpidiopsis varians* differs from that of Shanor (1939a) mainly in the ornamentation of the walls of zoosporangia and resting spores. In the material described by him there is much variation in the ornamentation of the wall of zoosporangia. They range from being perfectly smooth-walled to those densely covered with sharp spines with varying intermediates. The sporangia in the present isolates are mostly smooth-walled and rarely with fine echinulations and that too only on some of those growing in *Achlya proliferoides*. These differences are, however, not of much significance as Shanor (1939a 1939b) and McLarty (1939, 1941a) have already reported that even in monosporangial cultures there can exist wide range from perfectly smooth to densely spiny condition of zoosporangial wall.

The exspores of resting spores, which are mostly continuous with those of companion cells, assume various configurations. The mature resting spores in the hyphae of *Achlya proliferoides*, are frequently surrounded by broad based spines which are joined at their bases to form a reticulum (Figs. 8, 14). These are similar to those described for *O. fusiformis* and *O. minor*. Eoxspores on some bear long tapering spines similar to those described for *O. saprolegniae* (Fig.11). Some resting spores have undulating exospores like those described for *O. major* and *O. incrassata* (Figs. 9 and 10). Thin warty exospores looking like those described for *O. vexans* are also not uncommon (Fig, 12). There are other variations also in the ornamentation of exospore (Figs. 8, 13, 15). Variations have also been noted in the exospore structure of resting spores of fungus growing in the hyphae of *Isoachlya anisospora* var. *indica* (Figs. 3, 4, 5, 6 and 7). Shanor (1939a) also recorded some variation in the sharpness of the spines of the exospore wall on the resting spores of *O. varians* studied by him. McLarty (1941a) has reported similar variation in exospore structure of resting spore of *O. achlyae*.

The present study extends the host range of *O. varians* to *Isoachlya*.

SUMMARY

Olpidiopsis varians has been recorded for the first time from India, parasitising *Isoachlya anisospora* var. *indica* and *Achlya proliferoides*. Of the two hosts, the former is the first species of *Isoachlya* reported to be infected by this parasite and the latter is a new natural host species.

The variation in the structure of exospore of resting spores (including also the companion cells) indicates that it is not a suitable diagnostic character upon which specific distinctions can be made.

ACKNOWLEDGEMENTS : The author wishes to express his gratitude to Dr. K. S. Bhargava, Head of the Botany Department, University of Gorakhpur, for his guidance and encouragement during the course of this investigation. He is thankful to Mr. V. M. Chakco, Principal, St. Andrew's College, for providing facilities and to his colleague Mr. F. Abbasi for his help in the preparation of figures.

Department of Botany,
St. Andrew's College,
University of Gorakhpur,
Gorakhpur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

McLARTY, D. A. (1939) Observations on the genus *Pseudolpidium*. *Amer. J. Bot.*, **26** : 194-195.

McLARTY, D. A. (1941) Studies in the family Woroninaceae. 1. Discussion of a new species including a consideration of the genera *Pseudolpidium* and *Olpidiopsis*. *Bull. Torrey Bot. Cl.*, **68** : 49-66.

SHANOR, L. (1939a) Studies in the genus *Olpidiopsis*. I. Resting spore germination in a new species. *J. Elisha Mitchell Sci. Soc.*, **55** : 167-177.

SHANOR, L. (1939b) Studies in the genus *Olpidiopsis* II. The relationship of *Pseudolpidium* Fischer and *Olpidiopsis* (Cornu) Fischer. *J. Elisha Mitchell Sci. Soc.*, **55** : 179-195.

SHANOR, L. (1940) Studies in the genus *Olpidiopsis*. III. Some observations on the host range of certain species *J. Elisha Mitchell Sci. Soc.*, **56** : 165-176.

SPARROW, F. K. Jr. (1960) *Aquatic Phycomycetes*, University of Michigan Press, Ann Arbor.

SRIVASTAVA, G. C. AND K. S. BHARGAVA (1963) Observations on Indian Aquatic Fungi. I. Three species of *Olpidiopsis* from Gorakhpur. *Indian Phytopath.*, **16** : 271-274.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

A note on estimation of losses due to leaf blight of wheat caused by Alternaria triticina.—V. V. CHENULU AND AMAR SINGH, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—Wheat is the most important cereal providing staple food for millions of people all over the world. The area under wheat in India at present has been estimated to be 30 million acres and the production of grain at about 8.8 million tons. *Triticum vulgare*, the bread wheat, and *T. durum*, the durum wheat, are the two species most commonly cultivated in the major wheat growing areas of the Punjab, Uttar Pradesh, Bihar, Northern Rajasthan, the Central and Peninsular India. A third species, *T. dicoccum*, is also grown to a small extent in Nilgiri hills, Mysore, Andhra Pradesh and Bombay.

A disease causing severe blighting of leaves has been observed to attack mainly the durum wheats and the hybrids having durum "blood". The disease is responsible for considerable loss in yields in parts of Maharashtra, Bihar and Bengal. The symptoms in early stage of infection comprise of brown oval spots on the leaves which become irregular as they enlarge, sometimes accompanied by a bright yellow marginal zone around the spots. As the disease advances, several spots coalesce covering the leaf partially or completely giving it a blighted appearance. Heavily infected fields present a scorched appearance and can be recognised from a distance.

The disease was first reported and described from India by Prasada and Prabhu[2] and the pathogen identified as *Alternaria triticina*. Detailed investigations on symptoms, etiology, host-parasite relationship, factors influencing disease development, cultural characters of the pathogen, host-range, varietal resistance and survival of the pathogen from season to season etc. have been made (Prabhu[1], unpublished thesis). Although the disease has been reported to be very destructive, and is a limiting factor in wheat cultivation (particularly the *durum* wheats) in many parts of India no precise estimates on the losses inflicted by the disease are available. Preliminary investigations were, therefore, undertaken by the authors with a view to finding out the disease intensity/yield loss relationship of *Alternaria* blight of wheat. The results obtained are reported in this paper.

Three susceptible varieties of wheat *viz.*, Kenphad 21, Kenphad 32 and N. P. 830 were used in these studies. The seeds were sown in 10" pots filled with sterilized soil in the month of December, 1963. Inoculations were made at three stages of crop growth *viz.*, 2, 2½ and 3 months after sowing. First inoculation was made in the month of February, 1964 when the plants were 2 months old and the prevailing temperature 25°C was optimum for infection. Inoculation of juvenile plants (younger than 2 months) was not attempted as they have been found not to take

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

infection (Prasada and Prabhu[2]). The inoculum used for infection comprised of a spore suspension of *A. triticina* obtained from 21 days old cultures on standard nutrient agar medium. It was prepared by scraping the spores and mycelium from the agar surface and adding sterile distilled water. In order to get a homogenous spore suspension, the latter was churned in a waring blender for 10 minutes and strained through cheese cloth. The leaves were sprayed thoroughly with this spore suspension. The inoculated plants were kept in a humid chamber for 48 hours after which they were kept on green-house benches. Typical symptoms were noticed on the 4th or 5th day following inoculation. The disease intensity on individual plants was measured 10 days after inoculation when symptom development was complete. The infected leaves were given numerical ratings of 0, 1, 2, 3 and 4, taking into consideration the leaf area covered by the spots. Thus leaves with no sign of infection received a score of 'O' while these with highest infection where the entire leaf was fully covered with spots and had dried up received a score of '4'. The infection index of individual plants was calculated by the formula given below :

$$\text{Infection index} : \frac{\Sigma \text{ class rating} \times \text{class frequency} \times 100}{\text{Total no. of leaves} \times \text{Maximum rating}}$$

On an average, 10-20 plants were used for measuring the infection index of each variety when inoculated at different stages. The individual plant yields and also average yield of each variety in different treatments was recorded. The results are presented in Table 1.

TABLE 1 : Loss in yield of different wheat varieties due to infection by *A. triticina* when infected at different stages of crop growth

Wheat Variety	Control			1st inoculation (Two month old seedlings)			2nd inoculation ($2\frac{1}{2}$ month old seedlings (Boot-stage)			3rd inoculation (3 month old seedlings)		
	1*	2*	3*	1	2	3	1	2	3	1	2	3
Kenphad 21	0	483	0	46	140	72	62	68	85	75	222	54
Kenphad 32	0	2054	0	35	152	92	49	46	98	76	511	75
N. P. 830	0	532	0	45	198	87	66	16	99	65	436	71

*1 = Infection index.

*2 = Average yield of grain per plant in milligrams.

*3 = Per cent loss in yield.

From the data in Table 1, it is evident that the loss in yield of grain due to *Alternaria* blight of wheat varies from variety to variety and again within a variety the growth stage at the time of inoculation is a factor influencing

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

loss in yield. Losses, however, are maximum when plants get infected at 2½ month stage (Boot stage). The losses, however, in the three varieties are comparatively less if infection takes place after ear emergence (3 months growth stage) and grain setting has started. The infection index was least in the first inoculation and almost constant in the second and third inoculations. Thus the boot stage appears to be the most critical stage and losses are maximum if infection occurs at this stage.

ACKNOWLEDGEMENTS : We are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, for his interest and encouragement. Our thanks are also due to Dr. R. Prasada and Dr. M.M. Payak for critically going through the manuscript and helpful suggestions.

[1]Prabhu, A. S., *Leaf blight of wheat caused by a new species of* Alternaria. Thesis for the Ph. D. degree of I. A. R. I., (1962).

[2]Prasada, R. and A. S. Prabhu, *Indian Phytopath.*, **15** : 292-293, (1962).

A new Bacterial Canker of Carissa congesta in Maharashtra :—

L. MONIZ, J. E. SABLEY AND W. D. MORE, Plant Pathology Laboratory, College of Agriculture, Poona-5—A new bacterium inciting cankerous spots on "*Karvanda*" (*Carissa congesta* Wight) was observed during the Monsoon of 1962 in Poona District of Maharashtra State. The symptoms of the disease are initally manifested on the underside of the leaves as brown corky spots measuring 4-5 mm. in diameter, surrounded by clear 'halos'. These cankerous spots increase in size and the central portions get raised with a deposition of the bacterial exudate. As the disease advances, the spots get distributed throughout the upper and lower surfaces of the leaves bringing about coalescence of several spots. With the advance of the disease, the stems and tender twigs also get affected.

The technical description of the pathogen is being given below :

Xanthomonas carissa sp. nov. (*Etym.-carissa* affecting leaves of *C. congesta*)

Short rods with rounded ends, single but rarely in pairs, measuring $2.5 \times 0.66\mu$ Gram negative, capsulated, non spore-forming, not acid fast; motile, with a polar flagellum, colonies on potato dextrose agar flat, circular with entire margin, smooth, glistening, butyrous, 14 mm. in 8 days, lemon chrome (R^1); growth on potato cylinder copious, flowing, lemon chrome (R); gelatine and starch attacked; milk peptonized with curdling; litmus reduced; no lipolytic activity; NH_3 and H_2S from peptone, NO_3 not reduced, no indole; M.R. and V.P. tests negative; in a peptone free medium acid without gas from dextrose, arabinose, xylose, lactose, galactose and mannitol; no growth on dulcitol; aerobic; thermal death point about 51°C., optimum temperature for growth 25°-29°C.

Pathogenic to *Carissa congesta* Wight, Poona, Maharashtra State.

A detailed account will be published elsewhere.

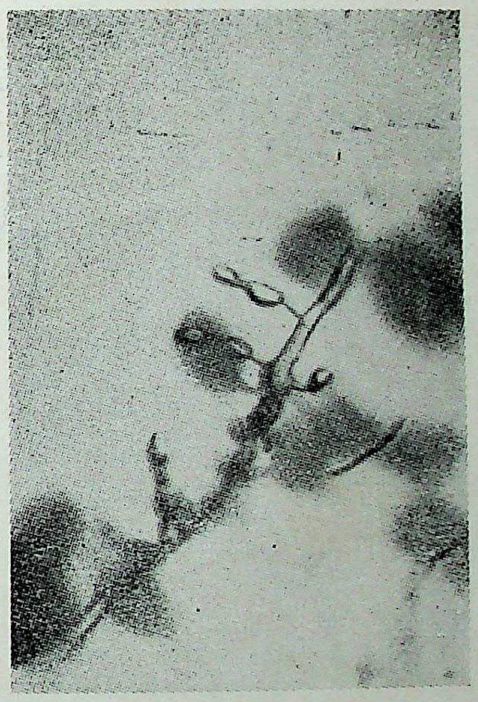
[1]Ridgway, R. (1912) *Colour standards and colour nomenclature.* Illus. Washington D.C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Formation of secondary sporidia in Uromyces decoratus Syd.—L. M. JOSHI, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—The teleutospores of *Uromyces decoratus* Syd., (sunnhemp rust) germinate readily without any resting period. The material collected from the fields in the months of January or February in Delhi germinated in less than 48 hours at a temperature range 18-28°C (optimum being 23-25°C).

It has been reported earlier by Joshi[3] that the teleutospores sometimes show a tendency to produce secondary sporidia, each smaller in size than the previous one, while still attached to the promycelium. At times, a continuous chain of 3-4 sporidia may be found. The chain, however, is very delicate and with slightest pressure on the coverslip or disturbance in water film, the sporidia separate out. Arthur[1] has mentioned that the formation of the secondary sporidia in rusts was reported by de Bary and Tulasne. Bessey[2] also recorded that under certain conditions of humidity, the primary and secondary sporidia of *Kunkelia nitens* (Schw) Arthur, remained attached in chains of four or five. Thirumalachar[4] has recorded the occurrence of secondary and tertiary sporidia in *Uromyces hobsoni* Vize.



It was observed that the material collected in the months of October or November took much longer time (upto 7-8 days at 23-25°C) to germinate. However, the material collected from the same field in January or February, and as such subjected to natural conditions for 3-4 months, germinated readily in less than 48 hours and sometimes also showed budding of sporidia. Since the formation of secondary sporidia was observed only in old material, effect of two factors *viz.*, storage

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and uncongenial temperatures at the time of germination, was studied in the first instance. It was found that 6-8 months old material stored in refrigerator at 5°C at times showed relatively higher percentage (1-2 per cent) of such germinating spores as compared to freshly collected material (collected in January or February) which usually showed normal type of germinating spores. The percentage of the former type of germinating spores was much more (sometimes 15-20 per cent) if the stored material was germinated either at the maximal (26-28°C) or minimal (18-20°C) temperature ranges. The abnormalities in germination of teleutospores are possibly best expressed if the material is stored for a long time at low temperatures and germinated at extreme temperature ranges.

During the course of these studies a few other abnormalities in teleutospore germination were also observed. In one case, a single spore produced two promycelia which emerged out from the apical end. Both the promycelia, however, did not produce sterigmata. Apart from these, quite often some teleutospores produced abnormally long promycelia having only rudimentary sterigmata without sporidia. Such structures were, however, only few.

ACKNOWLEDGEMENT : My grateful thanks are due to Dr. R. S. Vasudeva, former Head of the Division of Mycology and Plant Pathology and Dr. R. Prasada for their helpful suggestions.

[1]Arthur, J. C. *The Plant Rusts*, John Wiley and Sons, London. (1929).

[2]Bessey, E. A. *Morphology and Taxonomy of Fungi*, Blakiston Co., Philadelphia, U.S.A. (1950).

[3]Joshi, L. M. *Indian Phytopath.*, **13** : 90-96, (1960).

[4]Thirumalachar, M. J. *Phytopathology*, **29** : 783-792, (1939).

A Fusarium sp. mycoparasitic on stem rust of wheat, Puccinia graminis var. tritici (Pers.) Eriks. and Henn.:—R. PRASADA AND S. K. SHARMA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—Mycoparasites on wheat rusts have been reported by many workers, both from India and other countries. During the maintenance of rust cultures at Delhi, it was observed that sometimes, when the humidity in the glass house was high (above 60 per cent), the uredinial pustules of black rust of wheat (*Puccinia graminis* var. *tritici*) were covered with a whitish mycelium, which was different from the hyperparasites earlier reported by Prasada[3] and Sreekantiah and Joshi[4]. This mycelium was isolated in pure cultures and the fungus identified as *Fusarium*, sp. Chester[2] reported the occurrence of *F. nivale* on the uredinial pustules of *Puccinia triticina* on overwintering and snow-covered wheat in Europe. The *Fusarium* sp. isolated here did not infect *Puccinia triticina* spores and it was virulent and profusely sporulating at higher range of temperatures (25°-28°C) and often failed to appear at lower temperatures (below 17°C). Reviewing the literature on

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

nature of mycoparasites, Barnett[1] observes that "the details of this relationship have not been thoroughly studied". This aspect, has, therefore, received a closer attention in our studies reported here.

The fungus was characterized by the presence of hyaline rosy mycelium which changed to dark colour; spindle to oval shaped micro-conidia, sickle shaped, one to six septate macroconidia, tapering at both ends. These characters place the fungus in the genus *Fusarium*. A culture sent to Dr. William C. Snyder has been identified by him as *F. roseum* "Gibbosum."

To study the association of the fungus with black rust of wheat, a water suspension of *Fusarium* spores was sprayed on (i) wheat seedlings bearing well developed uredinial pustules, (ii) on healthy wheat seedlings in which the first leaves had unfolded and (iii) on wheat seedlings of same age, but slightly injured. Wheat seedlings having uredinial pustules of same age were sprayed with sterilized distilled water and kept as control. All the pots were transferred to humid chambers (60 per cent or above) at 25-28°C, under identical conditions. After 5-7 days, it was observed that only in the first treatment, a fluffy whitish growth of mycelium appeared. For further confirmation, fresh uredospore dust was kept on two dry, sterilized slides under aseptic conditions. Uredospores on one of the slide were mixed with *Fusarium* spores and transferred to a sterilized humid chamber, and the second slide was kept as such uninoculated in a separate moist chamber and incubated at 25°C. After 5-7 days, a whitish growth of mycelium was observed over the inoculated mass of uredospores, whereas no such mycelial growth was observed in the second case. These experiments clearly demonstrate that this fungus was only associated with the uredospores and had no affinity with their host (wheat seedlings).

In another experiment fresh uredospores alone and in mixture with *Fusarium* spores were kept for germination on separate slides. Whereas no germination of uredospores was observed in the mixture, there was a very high percentage of germination (80-90 per cent) in uredospores kept alone. When wheat seedlings were inoculated with a mixture of uredospores and spores of *Fusarium* or with uredospores collected from infected rust pustules, practically no rust infection was observed, thus indicating that the fungus adversely affected the germination of uredospores.

To study the relationship between *Fusarium* and uredospores, some uredospores from infected rust pustules and from uredospore mass, covered by the mycelial growth on slide, were mounted in glycerine after staining in cotton blue, separately. In both the cases, it was observed that *Fusarium* mycelium grows between the uredospores and some branches of mycelium enter in the uredospores through germ pores (Fig. 1). This intracellular mycelium is of various types, from single unbranched hyphae to branched mycelium; sometimes it occupies the whole cavity of spore. The infected uredospores appear to be empty.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

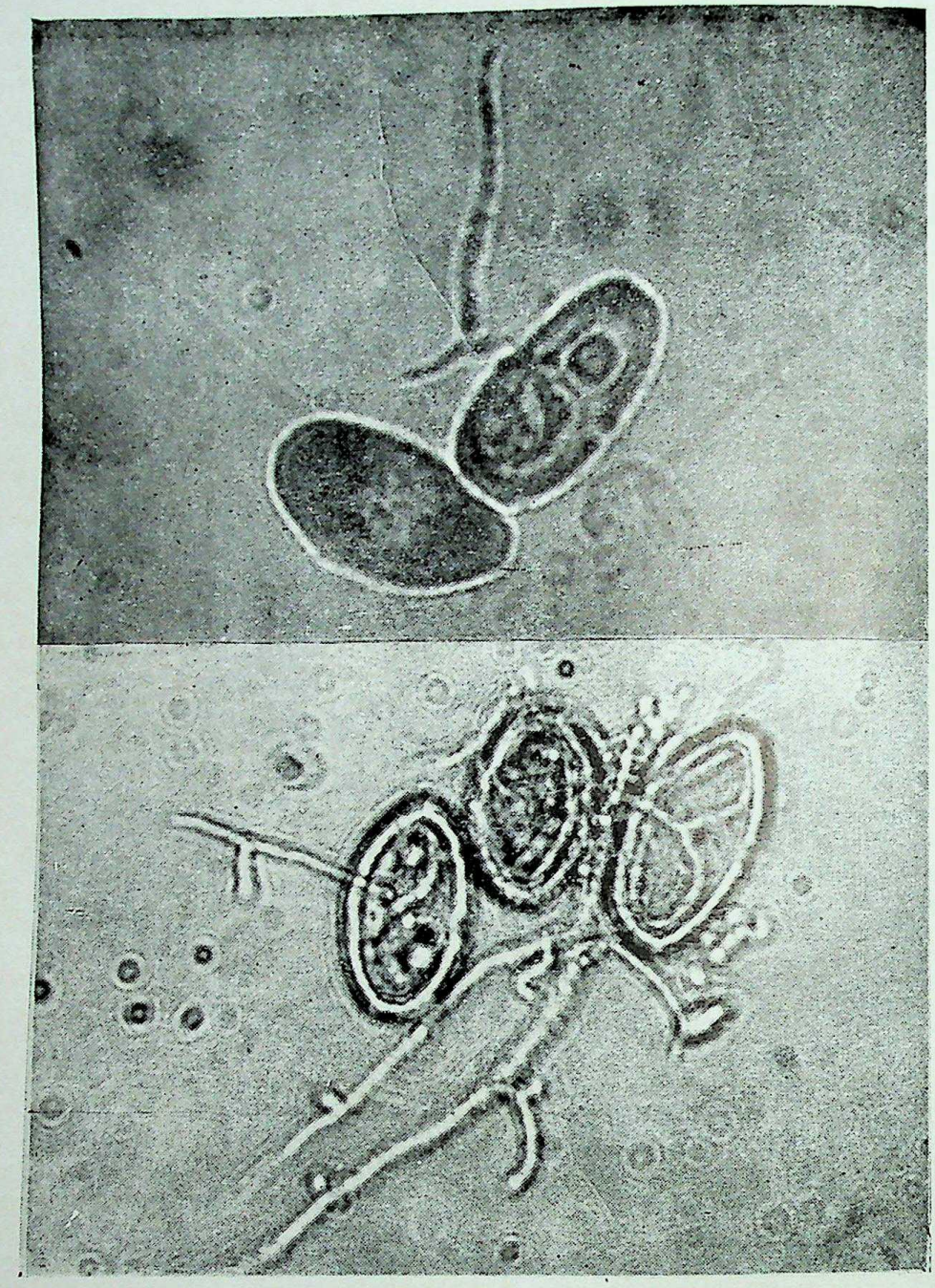


Fig. 1. *Fusarium* mycelium invading the uredospores through the germs pores.

From this, it is concluded that *Fusarium* mycelium absorbs its food material from uredospores by means of intracellular mycelium, which enter the uredospores through germ pores.

Considering that *Fusarium* is able to parasitise rust spores only when humidity is high (above 60 per cent) in the greenhouse, the use of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

this mycoparasite in the control of black rust in the field has a limited value. But the manner in which the *Fusarium* mycelium is able to enter the rust spores through the germ pores in order to obtain its nutrition, culminating in the death of the rust spores, is highly interesting.

Thanks are due to Dr. W. C. Snyder for the indentification of the fungus.

[1]Barnett, H. L., *Ann. Rev. Microbiol.*, 17 : 1-14, (1963).

[2]Chester, K. S., *The Nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat*. Chronica Botanica, Waltham, Mass. U. S. A., (1946).

[3]Prasada, R., *Curr. Sci.*, **17** : 215-216, (1948).

[4]Sreekantiah, K. R. and L. M. Joshi, *Indian Phytopath.*, **11** : 62-65, (1958).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ANNOUNCEMENT

INDIAN CURRENT SCIENCE ABSTRACTS

The need has been felt for a long time for a bibliographical periodical covering the entire scientific output of the country quickly and comprehensively. The Conference of Information Scientists convened by the Council of Scientific and Industrial Research in Mysore during 1963 realised this need acutely and directed the Indian National Scientific Documentation Centre (INSDOC) to take immediate action for ensuring bibliographical control of scientific publication in India.

INSDOC has decided to start a monthly abstracting service from January 1965, reporting under the title "Indian Current Science Abstracts" the work published by scientists in India. As far as possible efforts will be made to include scientific communications published abroad by Indian nationals. Original articles, short communications, critical reviews and informative articles, published in scientific and technical periodicals or in proceedings of scientific conferences, symposia, etc., monographs and other *ad hoc* publications, as well as patents and standards, will be noticed in this publication. The annual subscription for the publication will be Rs. 50/- only (Foreign £ 10 or $ 30).

This comprehensive coverage is possible only through the cooperation of various institutions, departments, sccieties and individuals who are bringing out scientific periodicals, reports, monographic publications etc. We appeal to all concerned to send us regularly such publications, including reprints of papers published in foreign periodicals, at the following address :

Indian Current Science Abstracts
Indian National Scientific Documentation Centre
Hillside Road, Delhi-12.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CIBA Pesticides

Dimecron — a systemic insecticide, based on Phosphamidon, is effective against sucking, chewing and mining insects. It is water-soluble, odourless, does not taint and most crops are free from residues ten days after treatment.

Nuvan — an insecticide with extremely potent fumigant and contact action against household and storage pests as well as against chewing and mining caterpillars on field crops.

Thiocron — an acaricide cum insecticide of low toxicity with specific action against mites.

Cuman — an organic fungicide with excellent rain resistance, suitable for the control of diseases on fruit trees and field crops.

Cosan — a wettable sulphur preparation of colloidal particle size for the control of powdery mildew on fruit and other crops. grey mildew on cotton. and mites.

Copramat — a fungicide, based on a combination of Copper-oxychloride and Ziram for the control of diseases on field crops.

C I B A

where research is the tradition

Stocks now available in India.
For detailed information, please write to
CIBA of India Limited, Pesticides Division, P.O.Box 479, Bombay 1

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

Perithecial production by different strains of Glomerella tucumanensis (Speg.) Arx and Mueller—*B. L. Chona, B. S. Bajaj and Rajeshwari Sharma* 241

Role of Vulpia myuros and Briza minor in the perpetuation of black rust of oats in the Nilgiri Hills—*L. M. Joshi and V. C. Lele* 245

Observations on Indian aquatic fungi—II. OCCURRENCE OF OLPIDIOPSIS VARIANS SHANOR AT GORAKHPUR—*G. C. Srivastava* 249

Phytopathological Notes

A note on estimation of losses due to leaf blight of wheat caused by Alternaria triticina—*V. V. Chenulu and Amar Singh* 254

A new bacterial Canker of Carissa congesta in Maharashtra—*L. Moniz, J. E. Sabley and W. D. More* 256

Formation of secondary sporidia in Uromyces decoratus Syd.—*L. M. Joshi* 257

A Fusarium sp. mycoparasitic on stem rust of wheat, Puccinia graminis var. tritici (Pers.) Eriks and Henn.—*R. Prasada and S. K. Sharma* 258

Announcement 262

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1964

President	S. SINHA
Immediate Past President	T. S. SADASIVAN
Vice-President	N. PRASAD
Secretary-Treasurer	S. P. RAYCHAUDHURI
Joint Secretary...	D. SURYANARAYANA

COUNCILLORS

Northern Zone	B. L. RENFRO
Mid-Eastern Zone	G. S. VERMA
Eastern Zone	U. N. MOHANTY
Central Zone	B. P. PATIL
Western Zone	K. G. NEMA
Southern Zone	S. B. LAL

EDITORIAL BOARD FOR 1964

R. PRASADA

Editor-in-Chief

S. P. RAYCHAUDHURI
M. J. THIRUMALACHAR
K. S. BHARGAVA
G. RANGASWAMI
KISHAN SINGH
D. N. SRIVASTAVA
M. M. PAYAK

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

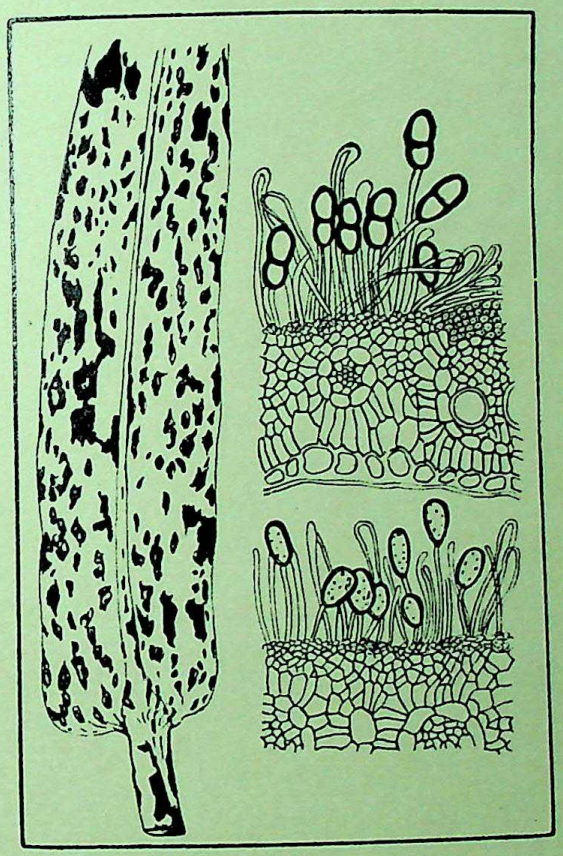
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVII **1964** *NUMBER 4*



PUBLISHED FOR

THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Respiration in plants under pathogensis—*T. S. Sadasivan* ... 263

Further studies on Phytophthora blight of sesamum—*P. D. Gemawat and N. Prasad* 273

Some physiological studies on sugarcane smut (Ustilago scitaminea Syd.)—*P. Appalanarasayya* 284

The nature of resistance of paddy to Helminthosporium oryzae Breda De Haan—*A. P. Mishra and Y. Prasad* ... 287

Mutation in colletotrichum falcatum Went. the causal organism of red rot of sugarcane--IV. MORPHOLOGICAL AND BIOCHEMICAL MUTANTS INDUCED BY IONIZING RADIATION—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* 296

A new pseudotorula from Rajasthan—*P. N. Mathur and G. Singh* 304

Studies on "Udbatta" disease of rice—*N. N. Mohanty* ... 308

Some interesting fleshy fungi from Himachal Pradesh—*H. S. Sohi, S. Kumar and P. K. Seth* 317

Occurrence of Tobacco mild mottle virus on tobacco—*H. N. Verma* 323

Evaluation of fungicides for the control of black rot of sweet orange—*Sushil Kumar and Rajendra K. Grover* ... 328

(Contd on. 3rd cover)

CG-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RESPIRATION IN PLANTS UNDER PATHOGENESIS[1, 2]

T. S. SADASIVAN*

The University Botany Laboratory, Madras-5

INTRODUCTION

Plant respiration as an index of disease has been the subject of investigation for a number of years. An increased respiration in tissues generally accompanies a variety of pathological conditions such as infection by fungi, bacteria and viruses (see Allen, 1953, Tables 1, 2; Uritani and Akazawa, 1959). Long before visible symptoms such as dechlorophyllation, abscission of leaves, leaf deformation, necrosis, wilting and death of plants and plant parts etc., become manifest, alterations in the respiratory pattern are discernible. This is not surprising since symptoms of disease are only end products of altered metabolism of invaded (or diseased) tissues and any such metabolic changes would have first repercussions on respiration one way or another. Therefore, study of the altered respiration of infected plants is of interest not only to phytopathologists but also to the plant physiologist and biochemist investigating the nature of the respiratory mechanism as well as the regulation of the respiratory rate. There is some evidence that genotypically controlled resistance to diseases depends on differences in metabolism, and respiration and respiratory rate as indices of such differences seem to open up new lines of interest both to the pathologist and plant breeder.

Prior to 1953, studies on respiratory stimulation in infected plants were confined mainly to the quantitative aspects of the increase. It was Allen (1953) who first pointed out that 'disease' was the result of disturbances in host metabolism induced by diffusible metabolites of the parasite. He postulated that increased respiration in mildewed leaves was the result of a toxin produced by the fungus which uncouples phosphorylation from electron transfer in host cells. Allen's paper focussed attention on the causes of respiratory increase in diseased tissues. Attempts have been made by several workers to isolate toxic agents inducing stimulation of respiration (Millerd and Scott, 1955, 1956; Farkas and Kiraly, 1955; Daly and Sayre, 1957; Bushnell and Allen, 1962; Uritani *et al.*, 1954). Subsequently, Allen (1954) suggested that stimulation of synthetic processes in the infected tissues could also be the cause of increased respiration. The involvement of new pathways in this augmented respiration has also been a subject of many investigations, particularly in rust infections.

---

[1]Presidential address delivered to the Indian Phytopathological Society at Calcutta in December, 1964.

[2]Memoir No. 4 from the Centre for Advanced Studies in Mycology and Plant Pathology.

*President of Indian Phytopathological Society for 1963.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The electron transport systems in infected tissues have been investigated in many diseases and this has raised the interesting point whether systems other than the cytochrome systems are of any importance in these tissues.

Some of the more interesting work on these and varied aspects of respiration of plants under pathogenesis including the results of the investigations carried out in our own laboratory will be considered here.

Quantitative changes and relation to resistance and susceptibility : Many workers have studied the relationship betewen respiration and resistance to plant pathogenic organisms. As early as 1912, Bach suggested that oxidative enzymes of the host might protect invaded tissues by oxidizing the toxic substances of parasitic origin.

From his extensive studies of the mildew of wheat, Sempio (1950) developed the concept of 'metabolic resistance' to parasitic attack. It would appear from his studies that it is the ratio between synthetic reaction like photosynthesis and breakdown reactions like glycolysis and respiration that decides the outcome of infection. Tissues having high rates of synthetic reactions coupled with low rates of breakdown reactions would be resistant to attack and *vice versa*. Sempio (*loc. cit.*) observed that when wheat plants were predisposed to attack by mildew by inducing wilting consequent upon sudden exposure to full sunlight for some hours the ratio between photosynthesis and glycolysis and respiration was shifted in the direction of susceptibility. In essence, Sempio's findings amount to this : if a resistant and a susceptible variety both have the same rate of photosynthesis, the resistant variety would probably possess a lower rate of respiration than the susceptible one.

Some interesting correlations between respiratory rate and resistance to parasitic attack have been observed in our laboratory.

Andal (1959) studied the respiratory rate of three varieties of rice differing in their susceptibility to *Fusarium moniliforme*. She found that the respiratory rates of both root and shoot tissues were directly proportional to the degree of susceptibility, the most susceptible variety showing the highest rates. Similar correlations were obtained by Lakshmanan (1956, 1959) in cotton varieties, resistant and susceptible respectively, to *F. vasinfectum*, the susceptible variety showing a higher rate of respiration than the resistant. Lakshmanan (1956) has further shown that there is a correlation between respiratory activity and induced resistance. When soil is amended with Zn, incidence of *Fusarium* wilt in cotton is found to be reduced (Sulochana, 1952). Lakshmanan (*loc. cit.*) observed that the respiratory rate of root and stem tissues of cotton plants grown in Zn-amended soil was lower than that of controls. It will be recalled that resistant varieties of cotton have a lower rate of respiration.

Studies by Rubin and his coworkers in the USSR (see Farkas and Kiraly, 1958) on potato-*Phytophthora*, cabbage-*Botrytis*, citrus-*Penicillium* and other diseases led them to conclude that resistant varieties respire

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

more intensively than susceptible ones and that the respiratory response to infection is of a greater magnitude in the resistant varieties. We have found this to be true in the case of groundnut plants in relation to their susceptibility to *Cercospora personata* (Swamy, 1964a). Of the commonly grown varieties, the variety TMV 2 was found to be most susceptible and the variety TMV 3 the least susceptible. Sporulation of the fungus is not as good on variety TMV 3 leaves as on those of variety TMV 2 (Shanta, 1957). Further, when the third leaves from below are inoculated, in variety TMV 3, the leaflets turn yellow rapidly and drop off while in TMV 2, the infected leaves remain attached for a longer time (Swamy, 1964a). Leaves of TMV 3 show a higher rate of respiration than those of variety TMV 2 (Swamy, 1964a).

The pattern of respiratory changes following infection is somewhat different in resistant and susceptible varieties and has been studied in several host-parasite combinations. In wheat infected by *Puccinia graminis* var. *tritici* in the resistant variety, a steep rise in respiration followed by a steep fall has been observed while in the susceptible variety, a steady rise in the respiration of infected leaves occurs (Samborski and Shaw, 1956). The situation is similar in barley leaves infected by powdery mildew (Millerd and Scott, 1956). In the roots of the susceptible cotton variety infected with *Fusarium vasinfectum*, Lakshmanan (1959) observed that a marked increase in respiration occurs only from the 14th day after inoculation. In the roots of the resistant varieties and of the susceptible variety rendered resistant by addition of Zn to the soil, even on the 7th day the infected plants show a higher rate of respiration than the healthy ones and the rate falls thereafter and approaches that of controls. In the stem tissues the situation was essentially similar to that in roots. Swamy (1964) has observed, again in *Cercospora personata*-infected leaves of groundnut variety TMV 3, a significant increase in respiration even on the 10th day after inoculation, while in the more susceptible variety TMV 2, such an increase was noticeable only from the 15th day after inoculation. The rise in respiration was more steep in variety TMV 3 than in variety TMV 2 and the respiratory peak was reached earlier in the former variety. Thus, essentially the same pattern obtains in infections by both obligate and facultative parasites.

Whereas plant tissues invaded by fungi or bacteria generally respond with an increased rate of respiration, in the case of virus infections, increase as well as depression of the respiratory rate have been observed. Owen's (1955, 1956, 1957) extensive work has brought out many interesting facts. An increase in respiration occurs in tobacco leaves within two hours after inoculation with tobacco mosaic virus indicating that increase in respiration does not depend upon active virus multiplication but is an immediate effect of entry of virus into the cells (Owen, 1955). Only under winter conditions an increase in respiration occurs in tobacco mosaic virus infected tobacco leaves. Increasing the light intensity during the period before inoculation decreases respiration rates after infection. The effect of extra light is negligible under conditions of high natural light. Further work by Owen (1956) has shown that in tobacco plants inoculated with tobacco mosaic virus the respiratory rate of inoculated leaves increases by a constant amount for 3 weeks

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

after inoculation and then decreases. The respiration of the younger non-inoculated leaves present at the time of inoculation is not affected at any time while that of leaves formed since inoculation is decreased by 10 per cent when they show symptoms. Unlike tobacco mosaic virus infection which increases respiration of inoculated leaves within an hour, infection by tobacco etch virus does not affect respiratory rate of inoculated or systemically infected leaves until they show external symptoms. The respiratory rate then rises above that of control leaves (Owen, 1957). In our laboratory, Ramadasan (1962) has studied the respiratory changes in leaves of *Dolichos lablab* directly inoculated with Dolichos enation mosaic virus (DEMV) and those systemically infected with the virus. In the directly inoculated leaves significant increase in respiration was noticed. In the systemically infected leaves also increase in respiratory rate occurred initially, but later a significant fall in the rate when compared to controls was seen. Conflicting results have been reported in the case of the respiratory changes in local lesion hosts of viruses. Yamaguchi and Hirai (1959) observed an increase in respiratory rate only after appearance of local lesions in *Nicotiana glutinosa* leaves inoculated with tobacco mosaic virus, while Ramadasan (1962), in our laboratory, reported an increased rate of respiration even before appearance of lesions in cluster bean leaves inoculated with DEMV. Merret's (1960) work has focussed attention on the need to express respiratory rate on a proper basis to get a correct picture of the effect of virus infection on respiration. In tomato plants infected with aucuba mosaic virus, the reduction in size of the plant is the result of reduction in cell size rather than in cell number. Internodes of healthy and virus-infected plants contain equal number of cells though the cells are smaller in size in the infected plants. On a fresh weight basis internodes from infected plant show a higher rate of respiration than healthy ones. On an internode basis the rate is lower in the infected tissue. Thus, virus infection results in a fall in respiratory ratio of individual cells. The results obtained on a fresh weight basis is due to the fact that unit fresh weight of infected tissue contains a larger number of cells (respiratory systems) than unit fresh weight of healthy tissue.

Stimulation of enzyme systems : The increase in respiration accompanying infection only reflects activation of enzyme systems connected with oxidative processes. There are many reports of increase in the activity of oxidative enzymes following infection. It has been suggested that oxidative enzymes presumably inactivate toxins thus conferring protection to tissues (Bach, 1912). Among enzymes known to be stimulated are polyphenoloxidase, ascorbic acid oxidase, catalase and peroxidase. Of these, polyphenoloxidase seems to play an important role in defence reactions.

Enormous increase in polyphenoloxidase activity has been observed in stem tissues of *Fusarium*-infected tomato plants (Sanwal, 1956). Kiraly (1959) has found polyphenoloxidase activity in leaf tissues of wheat varieties resistant to *Puccinia graminis* while no such activity was seen in the susceptible variety. Moreover, after infection, increased activity of the enzyme was observed in the resistant variety much earlier than in the susceptible variety where only in the final stages an increase occurred.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Polyphenoloxidase activity is considered to be involved in the hypersensitive reaction of resistant varieties. The oxidation product of phenols *viz.*, quinones are toxic to the host cell as well as to the pathogens and the death of the host cells invaded by the pathogen and of those surrounding the invaded cell arrests further progress of the pathogen especially in the case of obligate parasites. Polyphenoloxidase is believed to have a role in local lesion formation by viruses. Studies by Farkas, Kiraly and Solymosy (1960) have shown that in host-virus combinations wherever there is local lesion formation by viruses considerable increase in the polyphenoloxidase activity of the host tissues occur. This increase could partly account for the increase in respiration during local lesion formation. These workers have also shown that whereas there is an increase in polyphenoloxidase activity in *Nicotiana glutinosa* leaves inoculated with tobacco mosaic virus under conditions where local lesions are produced, at high temperatures, when this virus goes systemic in this host, no change in polyphenoloxidase activity is noticed.

Ascorbic oxidase activity also increases following infection in many diseases. Such an increase has been reported, for example, in wheat leaves infected by rust (Kiraly and Farkas, 1957) and in oats infected by *Helminthosporium victoriae* (Grimm and Wheeler, 1963).

The frequent reports of increased activity of enzymes like polyphenoloxidase and ascorbic oxidase in diseased tissues raise the question whether the increased respiration following infection is to be ascribed to the activity of these enzymes and if so what role cytochrome oxidase plays in diseased tissues. Daly and Jensen (1961) have recently shown that, in rusted hypocotyls of safflower, light-reversible inhibition of respiration by carbon monoxide occurs indicating an important role for cytochrome oxidase in 0_2 uptake. Further work seems to be, therefore, necessary before a correct assessment of the precise role of the other oxidases in respiration of diseased tissues can be made.

Alterations in pathways of respiration : Another interesting phenomenon that has come to light from a study of the respiratory metabolism of infected plant tissues is the alterations in respiratory pathways occurring as a result of infection in some diseases. For example, in infection by obligate parasites the greater respiratory activity accompanying infection is mediated through the pentose phosphate pathway instead of the glycolytic pathway, as shown by the considerable increase in the relative contribution of the first carbon of glucose to respiratory $C0_2$ in infected tissues and consequent decline in the so-called C6/C1 ratios measured with isotopes (Shaw and Samborski, 1957 ; Daly, Sayre and Pazur, 1957 ; see also Allen, 1959). In sweet potato tissue, infected with *Ceratocystis fimbriata*, however, with progress of disease there is an increase in C6/C1 ratio. In the initial stages of infection the ratio is equal in both cut and diseased tissues, but from 72 hours after inoculation there is a marked increase in ratio in infected tissues showing an increased operation of the glycolytic pathway (Akazawa and Uritani, 1962).

It is well known that the aerobic breakdown of the pyruvate produced from hexose is usually through the Krebs or TCA cycle in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

normal tissues. Changes in the operation of this cycle have been recorded in infected plants, mainly by studying the effect of malonic acid which inhibits an enzyme system (succinic dehydrogenase) in the cycle on oxygen uptake. A fall in the degree of inhibition of 0_2 uptake by malonate following infection has been reported frequently. While in healthy wheat leaves respiration is inhibited upto nearly 70 per cent, only 35-40 per cent inhibition is obtained in rusted and mildewed leaves (Kiraly and Farkas, 1955). Again, in potato tuber infected with *Gibberella saubinetii* inhibition by malonate falls considerably when respiration in the infected tissues is stimulated to a great extent (Verleur, 1960). In *Cercospora* infection of groundnut also, we have observed a lowered inhibition by malonic acid in the infected tissues, particularly in the later stages of infection (Swamy, 1964b). Whereas the observations referred to above are indicative of a lowering of the importance of the role of Krebs cycle in the oxygen uptake of diseased tissues, Akazawa and Uritani (1962) have recently shown that a more rapid turnover of the TCA cycle occurs as infection develops in the black rot of sweet potato.

Possible causes of respiratory increase : From what has been presented so far, it appears that in plants invaded by pathogenic organisms there is not only stimulation of respiration but also activation of enzyme systems and alterations in respiratory pathways. What brings about all these changes is not clearly understood. Nevertheless, it is well known that the availability of inorganic phosphate and phosphate acceptors play the most important role in the internal regulation of respiratory rate in plant and animal cells. The basis of this regulation is the fact that adenosine diphosphate (ADP) and inorganic phosphate are required as reactants both in the glycolytic sequence and in oxidative phosphorylation and glycolysis will be slowed down when the level of ADP falls (Beevers, 1961). Hence any reaction involving the rapid breakdown of adenosine triphosphate (ATP) and production of ADP would stimulate respiration. This can be brought about by synthetic reactions in the cell which use up ATP produced or, by what is called an 'uncoupling action' where the transfer of electrons takes place without formation of ATP from ADP. Uncoupling agents like 2, 4-dinitrophenol stimulate respiration by preventing phosphorylation of ADP. Both an uncoupling of oxidation from phosphorylation and the acceleration of synthetic processes in infected tissues have been suggested as the cause of respiratory stimulation. If the increase in respiration is due to either of these causes, an uncoupling agent like DNP would produce a lower response in the infected tissue than in the healthy.

In rusted wheat leaves (Shaw and Samborski, 1957), oat plants infected by *Helminthosporium victoriae* (Grimm and Wheeler, 1963) as well as in *Cercospora*-infected groundnut leaves (Swamy, 1964b) it has been observed that the stimulation by DNP is appreciably lower in the infected leaves (with a higher rate of respiration) than in healthy leaves. However, studies employing DNP alone can not decide whether the increased respiration in infected tissues is due to increased rate of ATP utilization in synthetic reactions or due to an uncoupling phenomenon. For this it is necessary to study incorporation of labelled phosphorus into ADP. But there is little work of this nature.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Toxins produced by pathogens have been implicated in the respiratory changes that are observed in infected plants. Culture filtrates of pathogens as well as toxic substances isolated from them have been shown to induce respiratory changes. Culture filtrates of several fusaria as well as that of *Cercospora personata* have been observed to stimulate respiration of plant tissues (Lakshmanan and Venkata-Ram, 1957 : Andal, 1959 ; Swamy, 1963). Some of the toxins like fusaric acid, α -picolinic acid, piricularin and victorin isolated from culture filtrates of pathogens like fusaria, *Piricularia* and *Helminthosporium victoriae* have profound effects on the respiration of host tissues (Tamari, Ogasawara and Kaji, 1963 ; Romanko, 1959). Fusaric acid (Andal, 1959 ; Sanwal and Waygood, 1961 ; Tamari *et al.*, *loc. cit.*), piricularin and α -picolinic acid (Tamari *et al.*, *loc. cit.*) inhibit respiration even in low concentrations. Victorin, on the other hand, stimulates respiration of the tissues of oat variety susceptible to *Helminthosporium victoriae* (Romanko, 1959). The stimulation of respiration by toxins of the pathogen could be the result of uncoupling of phosphorylation from electron transfer. But before any role is ascribed to a toxin in the augmentation of respiration following infection, the toxin has to be shown to be a vivotoxin.

As already mentioned, by increasing the turnover rate of ATP breakdown, synthetic processes can cause increased uptake of oxygen. There is some evidence of synthetic activities being accelerated following infection in some diseases (Shaw and Samborski, 1957 ; Daly and Sayre, 1957). In such cases at least part of the increase in respiration in infected plants can be ascribed to the stimulation of synthetic processes.

In summary, respiration as a phenomenon under pathogenesis needs further detailed investigation. There is little doubt that several enzyme systems are activated in the infected tissues, yet not all of these systems have been shown beyond doubt to be involved in electron transport *in vivo*. It is interesting that infection by rusts is invariably accompanied by a shift to the hexose monophosphate pathway whereas, in infections by facultative parasites such a phenomenon has not been recorded. In the case of infections by facultative parasites a distinct lacuna exists in our knowledge of the changes in the respiratory metabolism and detailed work, as done in rust infections, appears necessary. Moreover, studies employing labelled phosphate appear to be very urgently needed to elucidate the mechanism of respiratory rise in infected tissues.

References

AKAZAWA, T. AND I. URITANI (1962) Pattern of carbohydrate breakdown in sweet potato roots infected with *Ceratocystis fimbriata*. *Plant Physiol.*, **37** : 662-670.

ALLEN, P. J. (1953) Toxins and tissue respiration. *Phytopathology*, **43** : 221-229.

——————— (1954) Physiological aspects of fungus diseases of plants. *Annu. Rev. Pl. Physiol.*, **5** : 225-248.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

———— (1959) Metabolic considerations of obligate parasitism. In *Plant Pathology, Problems and Progress*, 1908-1958. Eds. Holton, C. S. *et al.*, Univ. Wisconsin Press.

ANDAL, R. (1959) *Studies on Foot-rot Disease of Rice.* Doctoral Thesis, University of Madras.

BACH, A. N. (1912) [Cited by G. L. FARKAS, AND Z. KIRALY (1958): Enzymological aspects of plant diseases. I. Oxidative enzymes. *Phytopath. Z.*, **31** : 251-272.]

BEEVERS, H. (1961) *Respiratory Metabolism in Plants.* Row Peterson and Company, Evanston.

BUSHNELL, W. R. AND P. J. ALLEN (1962) Induction of disease symptoms in barley by powdery mildew. *Plant Physiol.*, 37 : 50-59.

DALY, J. M. AND S. G. JENSEN (1961) Evidence for functional cytochrome oxidase in tissues infected by obligate parasites. *Phytopathology*, **51** : 759-765.

———— AND R. M. SAYRE (1957) Relations between growth and respiratory metabolism in safflower infected by *Puccinia carthami*. *Phytopathology*, **47** : 163-168.

———— , ———— AND J. H. PAZUR (1957) The hexose monophosphate shunt as the major respiratory pathway during sporulation of rust of safflower. *Plant Physiol.*, **32** : 44-48.

FARKAS, G. L. AND Z. KIRALY (1955) Studies on the respiration of wheat infected with stem rust and powdery mildew. *Physiol. Plant.*, **8** : 877-887.

———— AND ———— (1958) Enzymological aspects of plant diseases. I. Oxidative enzymes. *Phytopath. Z.*, **31** : 251-272.

————,———— AND F. SOLYMOSY (1960) Role of oxidative metabolism in the localization of plant viruses. *Virology*, **12** : 408-421.

GRIMM, R. AND H. WHEELER (1963) Respiratory and enzymatic changes in victoria blight of oats. *Phytopathology*, **53** : 436-440.

KIRALY, Z. (1959) On the role of phenoloxidase activity in the hypersensitive reaction of wheat varieties infected with stem rust. *Phytopath. Z.*, **35** : 23-26.

———— AND G. L. FARKAS (1955) Ueber die parasitogeninduzierte Atmungssteigerung beim Weizen. *Naturwissenschaften*, **42** : 213-214.

———— AND ———— (1957) On the role of ascorbic oxidase in the parasitically increased respiration of wheat. *Arch. Biochem. Biophys.*, **66** : 474-485.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

LAKSHMANAN, M. (1956) *Host-parasite Relationships in Plant Wilts with Special Reference to Respiration.* Doctoral Thesis, University of Madras.

——————(1959) Respiratory changes in *Fusarium*-infected cotton. *Phytopath. Z.*, **36** : 406-418.

——————AND C. S. VENKATA-RAM (1957) Influence of *Fusarium* culture filtrates on respiratory changes in cotton. *Proc. Indian Acad. Sci.*, **46** B : 131-137.

MERRET, M. J. (1960) The respiration rate of tomato stem tissue infected by tomato aucuba mosaic virus. *Ann. Bot.* N. S., **24** : 223-231.

MILLERD, A. AND K. SCOTT (1955) A phytopathogenic toxin formed in barley infected with powdery mildew. *Aust. J. Sci.*, **18** : 63-64.

————— AND ——— (1956) Host parasite relations in powdery mildew of barley. II. Changes in respiratory pattern. *Aust. J. biol. Sci.*, **9** : 37-44.

OWEN, P. C. (1955) The respiration of tobacco leaves in the 20-hour period following inoculation with tobacco mosaic virus. *Ann. appl. Biol.*, **43** : 114-121.

————— (1956) The effect of infection with tobacco mosaic virus on the respiration of tobacco leaves of varying ages in the period between inoculation and systemic infection. *Ann. appl. Biol.*, **44** : 227-232.

————— (1957) The effect of infection with tobacco etch virus on the rates of respiration and photosynthesis of tobacco leaves. *Ann. appl. Biol.*, **45** : 327-331.

RAMADASAN, A. (1962) *Studies on the Physiology of Virus-infected Plants.* Doctoral Thesis, University of Madras.

ROMANKO, R. R. (1959) A physiological basis for resistance of oats to victoria blight. *Phytopathology*, **49** : 32-36.

SAMBORSKI, D. J. AND M. SHAW (1956) The physiology of host-parasite relations. II. The effect of *Puccinia graminis tritici* Eriks. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. *Canad. J. Bot.*, **34** : 601-619.

SANWAL, B. D. (1956) Polyphenoloxidase and ascorbic acid oxidase activity in tomato plants infected with *Fusarium lycopersici*. *Proc. Indian Acad. Sci.*, **44** B : 257-270.

————— AND E. R. WAYGOOD (1961) Effect of fusaric acid on the oxidative phosphorylation of plant mitochondria. *Experientia*, **17** : 1-3.

SEMPIO, C (1950) Metabolic resistance to plant diseases. *Phytopathology*, **40** : 799-819.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SHANTA, P. (1957) *Studies on the* Cercospora *Disease of* Arachis hypogaea Doctoral Thesis, University of Madras.

SHAW, M. AND D. J. SAMBORSKI (1957) The physiology of host-parasite relations. III. The pattern of respiration in rusted and mildewed cereal leaves. *Canad. J. Bot.*, **35** : 389-407.

SULOCHANA, C. B. (1952) Soil conditions and root diseases. VII. Response of cotton plants to micro-element amendments and its relation to disease development. *Proc. Indian Acad. Sci.*, **36** B : 234-242.

SWAMY, R. N. (1963) *Studies on* Cercospora *Disease of Groundnut*. Doctoral Thesis, University of Madras.

———————— (1964a) Respiration in *Cercospora*-infected groundnut tissues. I. Respiratory stimulation following infection. *Phytopath. Z.*, **50** : 227-234.

———————— (1964b) Respiration in *Cercospora*-infected groundnut tissues. II. Effect of some enzyme inhibitors and 2,4-dinitrophenol on respiration. *Indian J. exp. Biol.* (In Press).

TAMARI, K., N. OGASAWARA AND J. KAJI (1963) Biochemical products of the metabolism of *Piricularia oryzae*. Paper presented at a Symposium on Rice Blast Disease at the International Rice Research Institute, Los Banos, Philippines (*In press* : Johns Hopkins Press, 1964).

URITANI, I. AND T. AKAZAWA (1959) Alteration of the respiratory pattern in infected plants. In *Plant Pathology* Vol. I. Eds. HORSFALL, J. G. AND A. E. DIMOND, Academic Press, New York.

———————— , ———————— AND M. URITANI (1954) Increase in respiratory rate in sweet potato infected with black rot. *Nature, Lond.*, **174** : 1060.

VERLEUR, J. D. (1960) Studies on the increased respiration of potato tuber tissue after infection with *Gibberella saubinetii* (Mont.) Sacc. *Acta bot. neerl.*, **9** : 119-166.

YAMAGUCHI, A. AND T. HIRAI (1959) The effect of local infection with tobacco mosaic virus on respiration in leaves of *Nicotiana glutinosa*. *Phytopathology*, **49** : 447-449.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FURTHER STUDIES ON PHYTOPHTHORA BLIGHT OF SESAMUM

P. D. GEMAWAT[1] AND N. PRASAD[2]

(Accepted for publication October 1, 1964)

Kale and Prasad (1957) showed that a serious blight disease of sesamum (*Sesamum orientale* L.) is caused by *Phytophthora parasitica* var. *sesami* Prasad. They have studied the morphological characters, host range, and the growth temperature relationships of the pathogen. Further studies on variation in pathogenicity, thermal death-point, soil temperature relationship of the causal organism and the role of seed in disease incidence and its chemical control were undertaken by the authors and the results are presented in this paper.

TABLE 1 : Length of lesions on stem produced by isolates of *P. parasitica* var. *sesami* on indigenous variety of *Sesamum*

S. No.	Isolate number	Length of lesion: Total length per pot containing 4 plants
1.	1	20.0 cms.
2.	2	17.5 ,,
3.	3	8.0 ,,
4.	4	7.5 ,,
5.	5	15.0 ,,
6.	7	17.0 ,,
7.	8	7.0 ,,
8.	9	7.0 ,,
9.	12	15.5 ,,
10.	13	4.0 ,,
11.	14	2.0 ,,
12.	15	4.0 ,,
13.	16	6.0 ,,
14.	17	2.5 ,,
15.	19	12.5 ,,
16.	20	3.0 ,,
17.	22	9.5 ,,
18.	Control	0.0 ,,

[1]Vijnan Mandir Officer, Dabok (Udaipur).

[2]Associate Dean, S. K. N. College of Agriculture, Rajasthan Agricultural University.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Variation in Virulence :

Isolations were carried out from diseased material of sesamum plants collected from different fields of the Institute and the adjoining villages. Seventeen isolates of *P. parasitica* var. *sesami* were used for inoculation purposes on the susceptible variety known as local Tali (white-seeded type) which is generally taken as a sole crop. Seeds were sown in sterilized pots containing sterilized soil. When the plants were one week old, they were thinned out so as to keep 4 plants per pot. Two pots were kept for each of the isolate tried. Two-week old plants were inoculated with the cultures of different isolates of the pathogen. For every pot, the culture of the pathogen grown on 10 c.c. of oatmeal agar was used, while the control pots were supplied with the blank oatmeal agar medium. Observations on stem lesions were taken daily, upto ten days of inoculation to record virulence of the isolates. The progress of the disease recorded after the tenth day of inoculation in terms of lesions on stem is represented in Table 1 ; it was observed that the lesions for different isolates varied from 2.0 to 21.0 cms. per pot, *i.e.*, 0.5 to 5.25 cms. per plant, suggesting thereby the existence of variation in virulence of different isolates of *P. parasitica* var. *sesami* (Fig. 2).

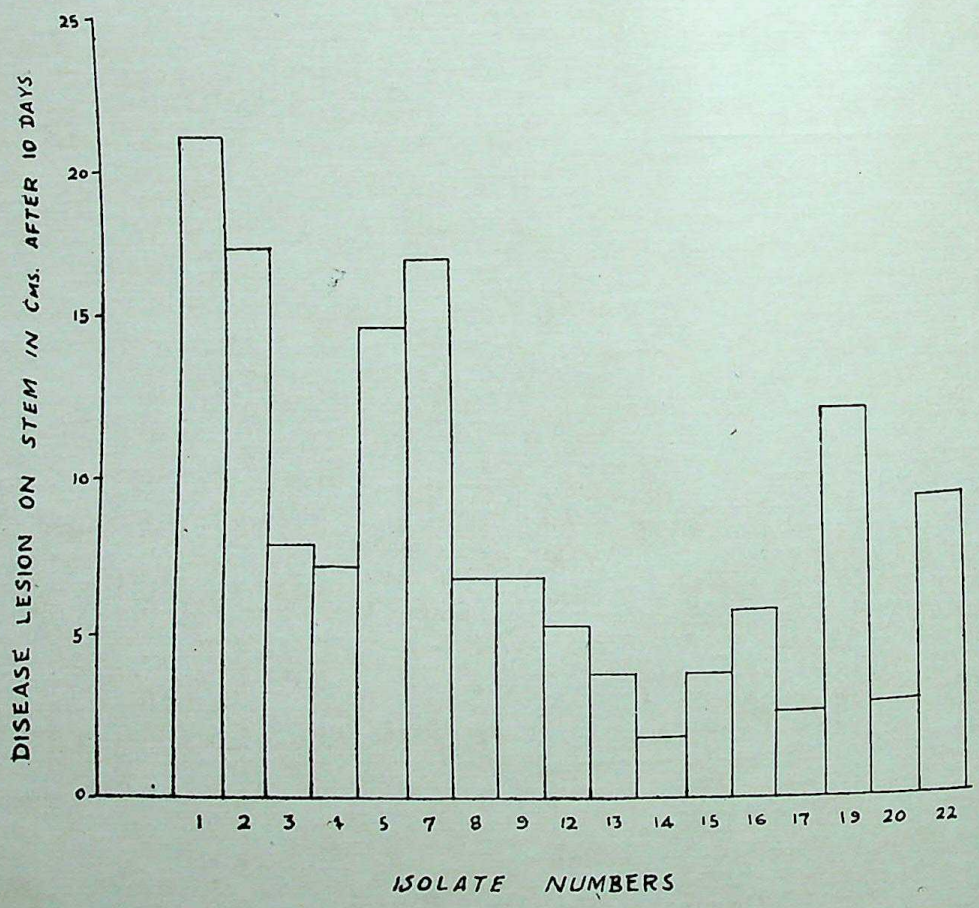


Fig. 1. variation in virulence of different isolates of *P. parasitica* var. *sesami*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THERMAL DEATH-POINT

The high degree of host specificity of *P. parasitica* var. *sesami* (Kale and Prasad, 1957) raised the important problem of the survival of the pathogen under varying conditions of climate encountered within this region. Earlier studies have shown the inability of the fungus to grow at temperatures higher than 35° C. In view of this the pathogen seems to be capable of survival during summer months. Experiments showed that the fungus could withstand in mycelial form temperatures up to 46° C. (thermal death-point being 47° C). Record of air temperatures during the months of April, May, and June for seven years showed that it had never gone above this level and rarely reached this figure. It is also likely that oospores may be able to withstand even higher temperatures. It seems reasonable to assume that the parasite can survive easily in the soil from one season to the other. There does not seem to be any difficulty for survival during the cold weather in this region, as cultures of the fungus kept in a refrigerator at 5° C. were found to be viable even after a lapse of one year.

Soil Temperature Relationship : The influence of the temperature was studied with regard to :— 1. The development of the disease. 2. The growth of the host plant. 3. The inter-relationship between the progress of the disease and the growth of host plant. 4. The growth of inoculum.

Five temperatures, *viz.*, 28° C., 30° C., 32.5° C., 35° C., and 37° C. were selected and were maintained in electrically-operated thermostatic controlled soil-temperature tanks. Galvanised iron pots, sterilized with 5 per cent formalin solution filled with double-sterilized soil and sown with seeds of local variety of sesamum were placed in temperature tanks after the seed had germinated. The seedlings were thinned out to keep 6 plants per pot. In all, 10 pots were kept for each temperature. When the plants were three weeks old, half of the pots were inoculated with 15-day old cultures, placing the inoculum one inch below the soil surface. The remaining pots served as controls. Immediately after inoculation, all the pots were watered twice daily.

The progress of the disease in each case was measured in terms of lesions in cms. and is recorded in Fig. 3 and is shown in Fig. 4. The disease symptoms were observed in plants growing at 28° C. temperature on eighth day. Further, this interval increased with the rise in temperature. The progress of the disease was also faster at lower temperatures *i.e.*, 28° C. and 30° C., while it became slower with the increase in temperature.

No marked effect of soil temperature on the growth of the host plant was noticed. The plants flowered practically at the same time in all cases (Fig. 5).

Comparative studies of temperature requirement of 4 isolates of *P. parasitica i.e.*, *P. parasitica* Dastur., *P. parasitica* var. *nicotianeae* Tucker; *P. parasitica* var. *sesami* and *P. parasitica* var. *piperina* Dastur. indicated that their growth was maximum at 30° C., they could tolerate a temperature of 35° C., but there was no growth at 37° C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

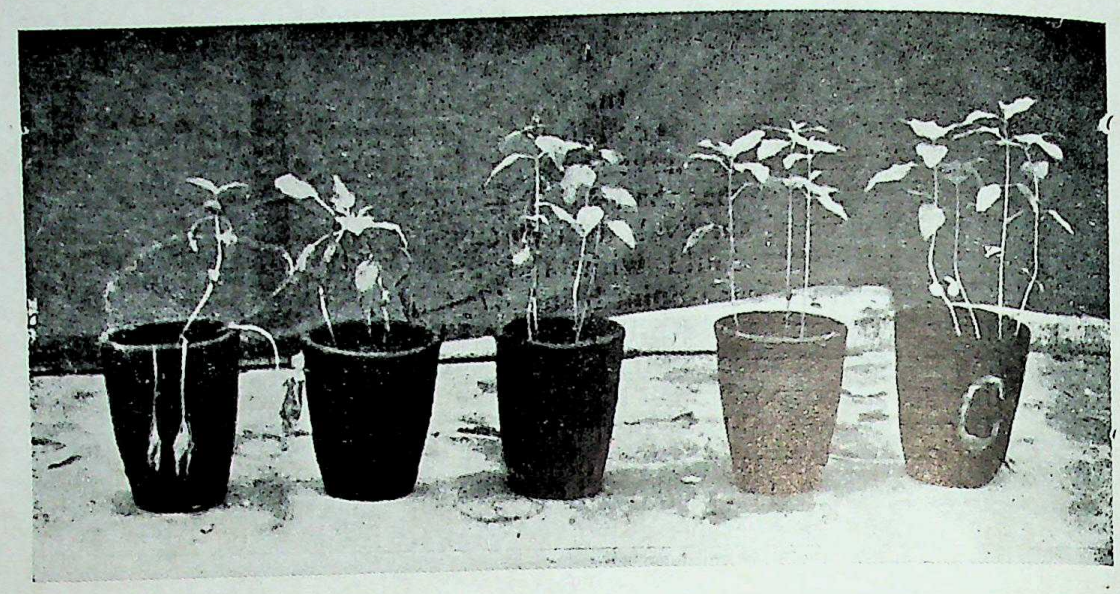


Fig. 2. Variation in virulence of different isolates of *P. parasitica* var. *sesami*.

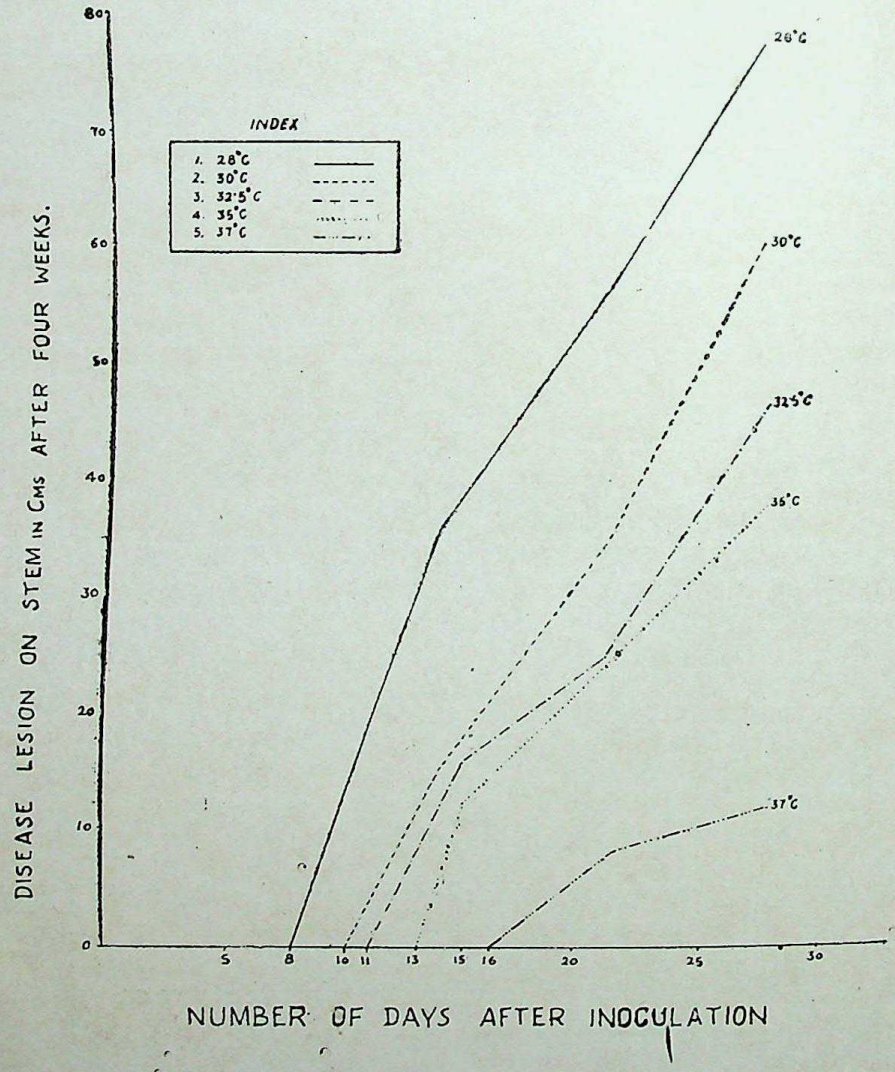


Fig. 3. The incidence of the disease at different soil temperatures

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

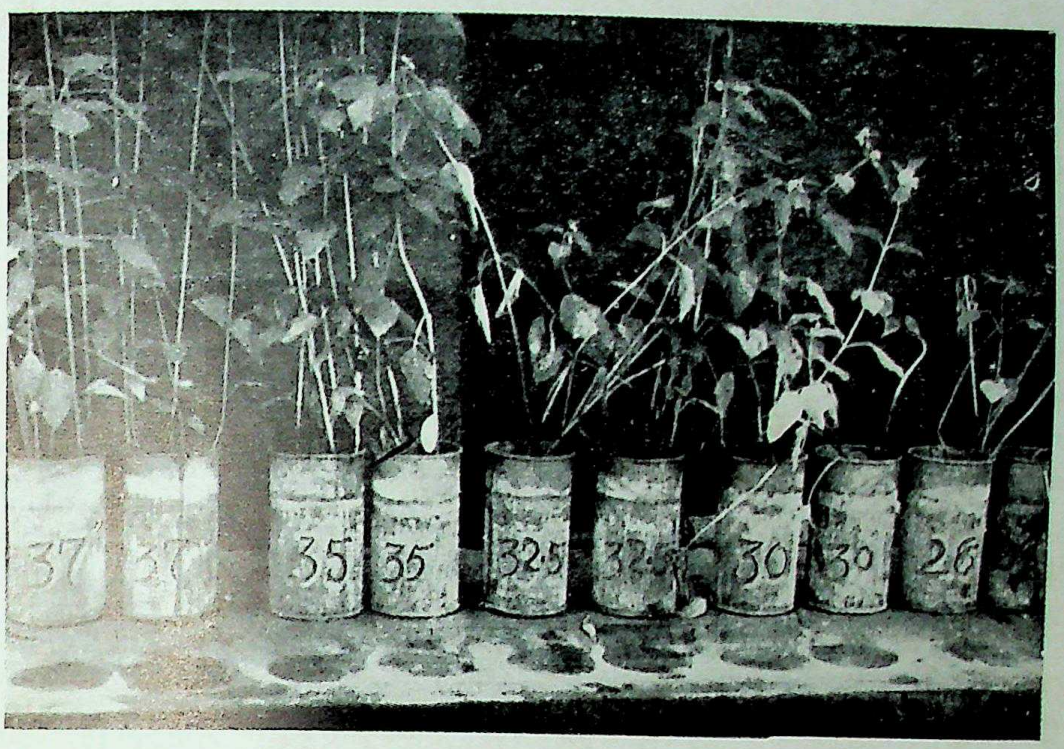


Fig. 4. The relation of soil temperature to the incidenee of the disease.

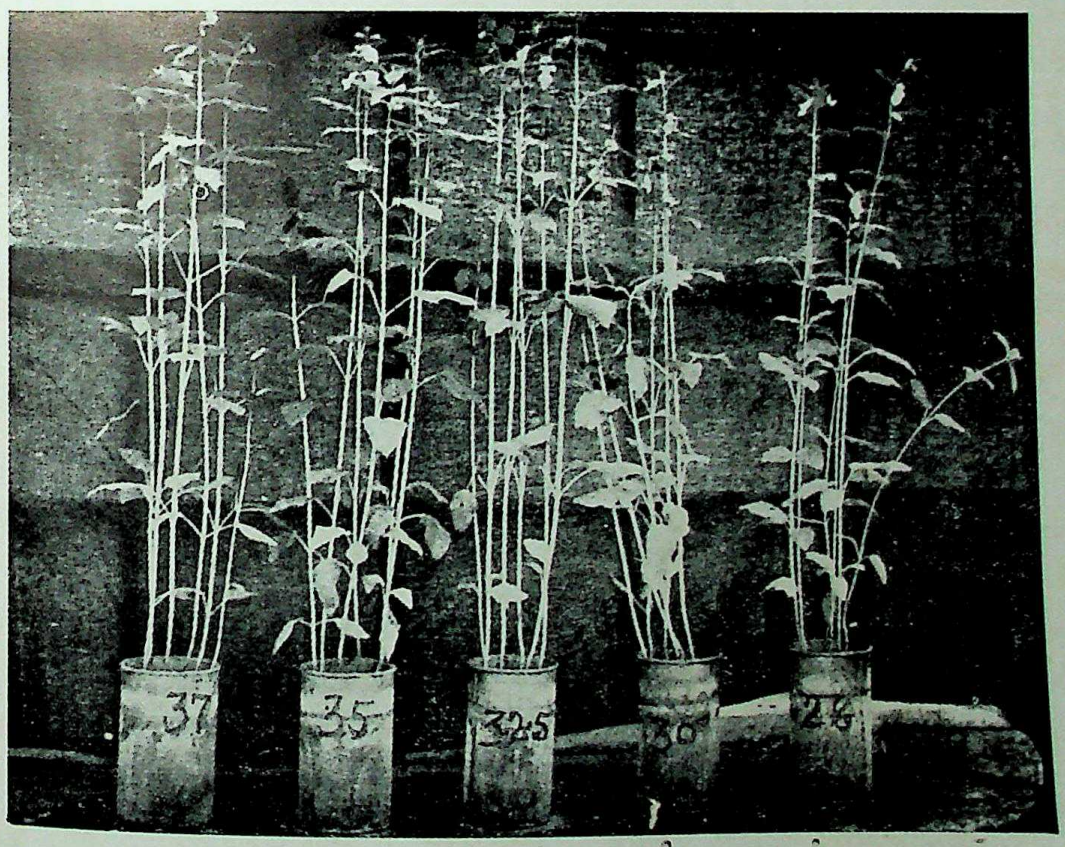
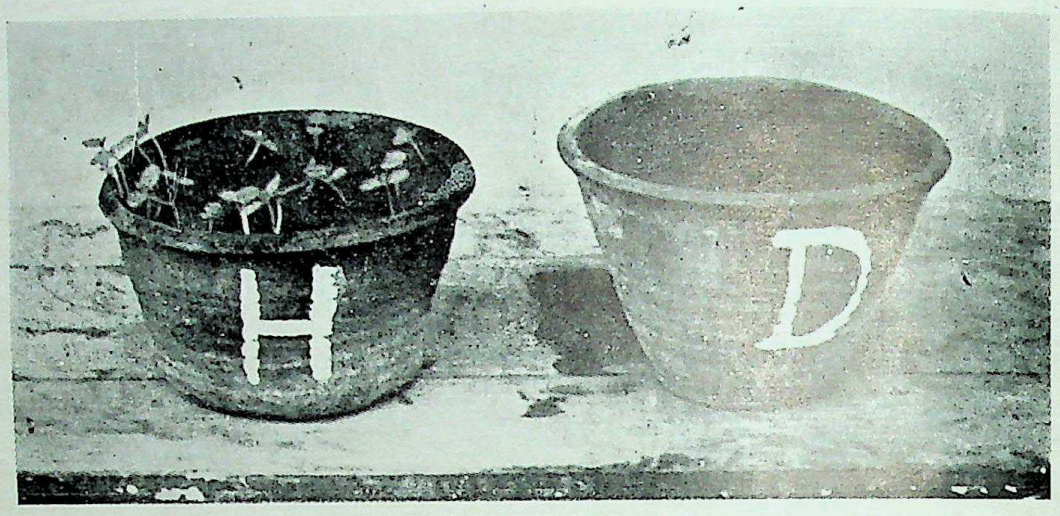


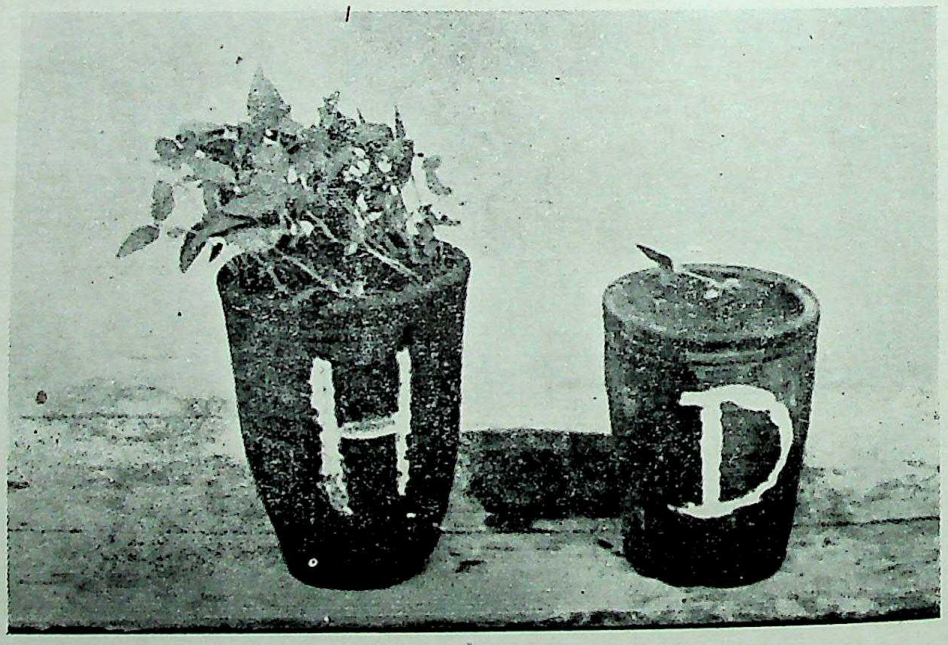
Fig. 5. The similar growth of sesamum plants at different soil temperatures.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Role of Seed in Disease incidence : Germination studies of seeds collected from healthy and infected pods revealed that the viability of seeds is reduced to a great extent due to the disease (Figs. 6 and 7.) However, experiments showed that the pathogen was not present within the seeds in viable form. It can, therefore, be concluded that this is not a seed-borne disease.





Figs. 6 and 7. Germination of seeds collected from healthy and diseased plants.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Chemical Control : Copper compounds have proved to be of value in the case of diseases caused by *Phytophthora* spp. Bordeaux mixture has been successfully used for the control of koleroga of Arecanut (*Phytophthora areceae*; Narsimhan, 1934); Late blight of Potato (*Phytophthora infestans;* Rieger, 1935), Betel-vine Wilt (*Phytophthora parasitica* var. *piperina;* Bombay Department of Agriculture, 1936). Similarly Zineb has also been used in controlling diseases caused by *Phycomycetes*. The efficacy of fungicides for the control of the sesamum blight was tested in the green-house.

An experiment was laid out with five fungicides, *viz.*, Bordeaux mixture 3-3-50, Bordeaux mixture 2-2-50, Perenox (Cuprous oxide) (4 ozs. in 10 gallons of water) and Zineb (Dithane-78) (2½ tea-spoonfuls per gallon). The plants were sprayed with different fungicides 3 days after inoculatlon, and every week thereafter.

TABLE 2 : Size of lesions produced by *P. parasitica* var. *sesami* after different fungicidal treatments

S. No.	Treatments	Total lesion in cms. 1st year	Percentage control	Total lesion 2nd year	Percentage control	Percent Average Control
1	2	3	4	5	6	7
1.	Bordeaux mixture (2-2-50)	40	84	51	73.1	78.5
2.	Bordeaux mixture (3-3-50)	34	86	12	93.6	90.0
3.	Cuprous oxide (Perenox)	49	80.4	28	85.3	82.8
4.	Copper oxychloride	64	74.4	56	70.5	72.5
5.	Zineb (Dithane-Z-78)	52	79.2	19	90	84.6
6.	Control	250	100	190	100	0.0

Observations regarding the length of the lesions produced on stems were recorded weekly and the final data taken 3 weeks after first spray. The experiment was repeated for two years (Figs. 8). The data are recorded in Table 2. The percentage control has been calculated as :

$$\frac{\text{Infection in control} - \text{Infection in treatment}}{\text{Infection in control}} \times 100$$

The disease can be controlled by spraying any of the five fungicides to the extent of more than 72.5 per cent. However, Bordeaux mixture (3-3-50) was the best, closely followed by Dithane Z-78. Three sprays of each at an interval of one week could quite efficiently control the disease (Photos 6, 7).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

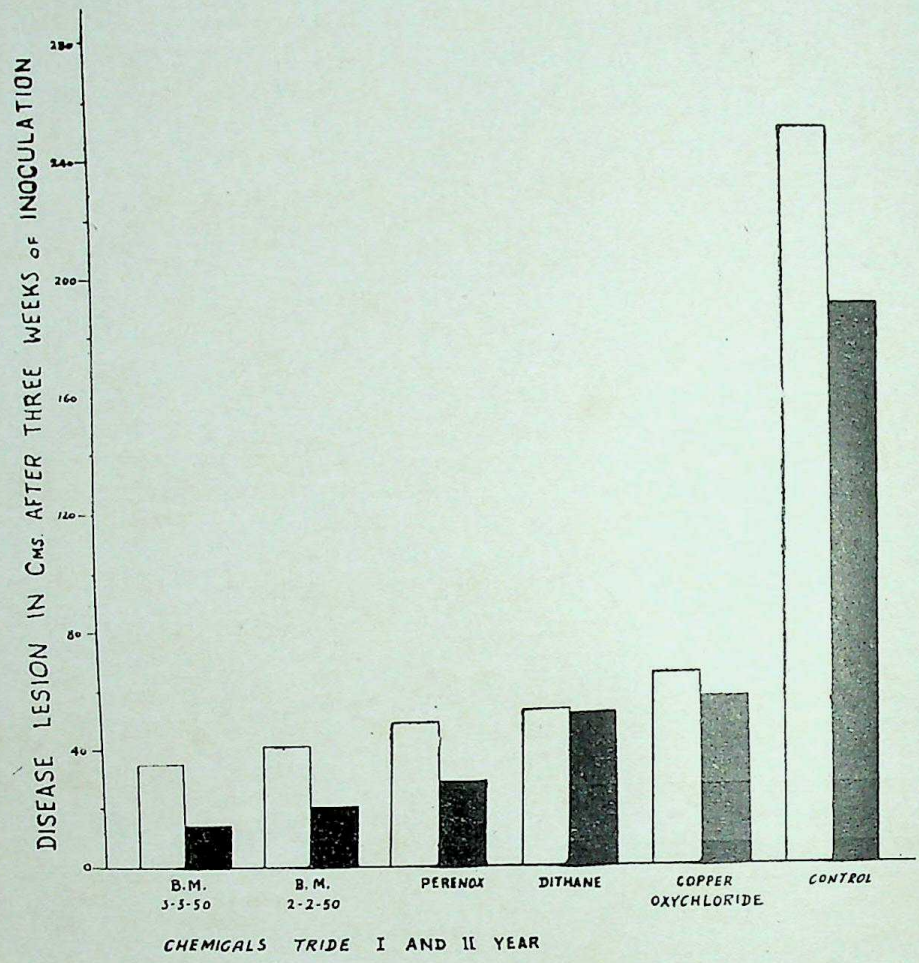


Fig. 8. The progress of the disease (*Phytophthora parasitica* var. *sesami*) after spraying different Fungicides.

Discussion

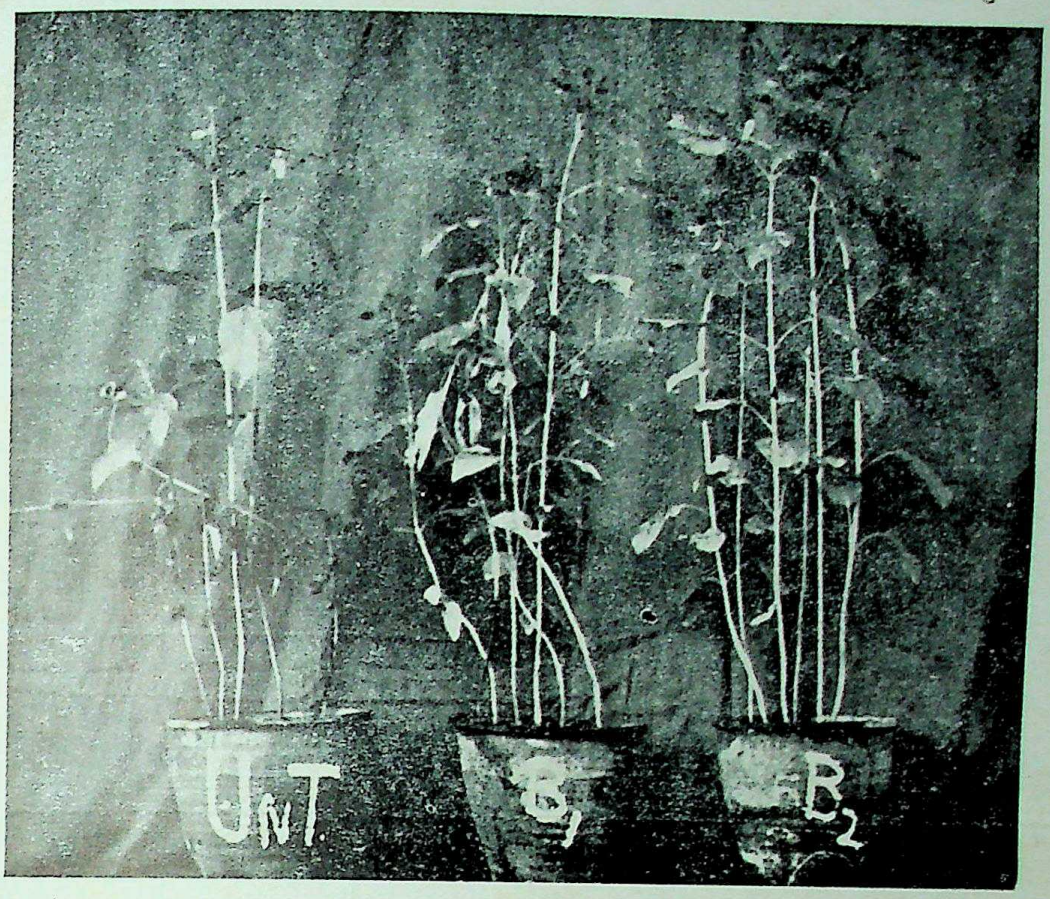
De Bruyn (1951) reported variation in pathogenicity of isolates of *P. infestans*. Tucker (1931) also observed pathogenic variation among isolates of *P. parasitica*. The existence of variation in virulence among the isolates of *P. parasitica* var. *nicotianeae* has been reported by Powers and Lucas (1952) and Appel (1954). The present work indicates that variation in incidence of the disease is possibly due to the existence of "strains" of *P. parasitica* var. *sesami* which differ among themselves in their pathogenicity.

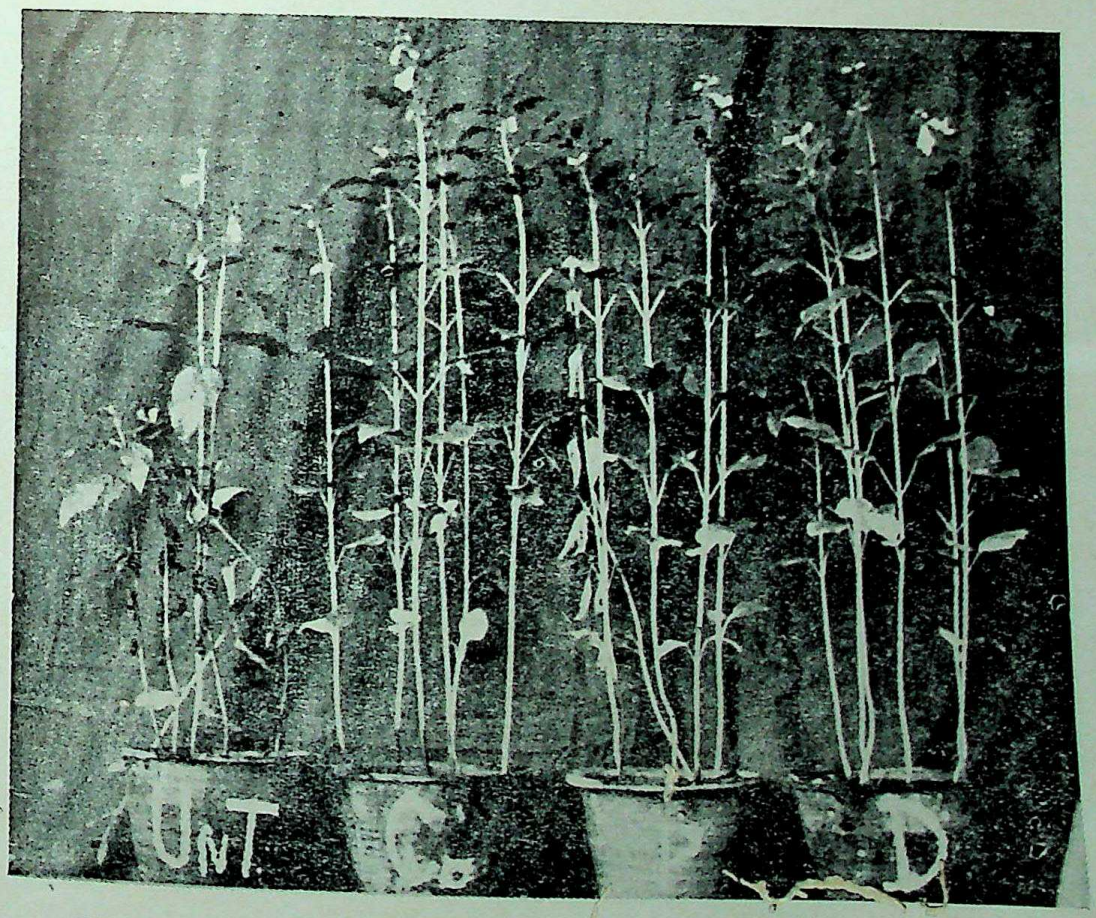
Studies on thermal death-point indicate that the pathogen can withstand temperatures prevailing both during the cold as well as the hot weather and that it can easily survive from one season to the other.

The importance of soil temperature relationships was recognised by Jones, Johnson and Dickson (1926). According to Tucker (1931), *P. parasitica* var. *nicotianeae* can grow at 35° C. Gratz and Kincaid

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri





Figs. 9 & 10. The incidence of the disease after spraying different fungicides. Untreated (UnT), Bordeaux mixture 2-2-50 (B_1), Bordeaux mixture 3-3-50 (B_2), Copper oxychloride (Co.), Perenox (P), and Dithane Z-78 (D).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(1935) have shown that the lowest temperature at which black shank (*P. parasitica* var. *nicotianeae*) appeared in transplanted tobacco is 16° C., the optimum being about 27° C., or 28° C; and the maximum temperature at which the disease symptoms appeared was 34° C. *P. parasitica* var. *sesami* was able to grow at 35° C. (Kale and Prasad, 1957), but ceased growth at 37° C. The optimum temperature of growth is 30°C. The present study indicates that the development of *Phytophthora* blight of sesamum is maximum at 28° C. and falls down with increase in temperature up to 37° C. The incidence of the black shank disease in tobacco shows direct parallelism to the growth of fungus at these temperatures. Similarly, in case of *Phytophthora* blight of sesamum, the incidence of the disease shows a close parallelism to the growth of the fungus. A close correlation between incidence of the disease to that of the growth of the fungus has also been observed by Uppal and Kulkarni (1937) in the case of sunnhemp wilt caused by *Fusarium vasinfectum* Atk. The different soil temperatures, *viz.*, 28° C to 37° C. have no effect whatsoever on the growth of the host plant, indicating that the difference in the incidence of the disease at different soil temperatures is the result of their influence on the causal organism.

Inability of infested seeds to produce any disease symptoms as such and their failure to reveal any growth of the causal organism establishes the absence of the pathogen within the seed in viable form, which shows that the *Phytophthora* blight of sesamum is not a seed-borne disease.

Rieger (1935) obtained striking results in the control of Potato blight (*P. infestans*) with two applications of Bordeaux mixture, the first being 1.5 per cent and second at 1 per cent after an interval of fortnight. Venkata krishnaiya (1950) could control *Phytophthora* blight of *Phaseolus vulgaris* Linn. by picking up infested pods and spraying plants with 1 per cent Bordeaux mixture, however, three applications of Bordeaux mixture (3-3-50) are necessary to control *Phytophthora* blight of sesamum, each at an interval of one week after the appearance of the disease.

SUMMARY

The present investigations suggest the existence of variation in pathogenicity of different isolates of *P. parasitica* var. *sesami*.

The pathogen can survive under the conditions obtaining in this region in spite of its restricted host-range.

The progress of the disease is rapid at 28° - 30° C. which falls down with an increase in temperature. The growth of sesamum plants remains unaffected at these temperatures.

The disease is not seed-borne. However, the viability of the seeds is reduced to a great extent due to infection.

The disease can be controlled with any of the five fungicides tried. Bordeaux (3-3-50) mixture was adjudged to be the most effective against the disease. Three sprayings of the fungicide are necessary to control it effectively.

Institute of Agriculture,
Anand.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

ANONYMOUS (1936) Control of Betel vine wilt. *Bombay Dept. of Agriculture* Leaflet No. 7.

APPEL, J. L. (1954) Variation in pathogenicity of *Phytophthora parasitica* var. *nicotianeae*. *Plant Dis. Reptr.*, **38** : 774-775.

DE BRUYN, H. L. (1951) Pathogenic differentiation in *Phytophthora infestans* (Mont) de Bary. *Phytopath. Z.*, **18** : 339-359.

*GRATZ, L. O. AND R. R. KINCAID (1935) Work in tobacco black shank in Florida. *Rept. Florida Agric, Exp. Sta. 1933-34, pp. 113-121.*

JONES, L. R., J. JOHNSON AND J. G. DICKSON (1926) Wisconsin studies upon relation of soil temperature to plant disease. *Res. Bull. No. 71, Agric. Exp. Sta. Univ. Wisconsin.*

KALE, G. B. (1954) *Studies on the blight of sesamum.* M. Sc. (Agri.) Thesis. Gujarat University.

KALE, G. B. AND N. PRASAD (1957) *Phytophthora* blight of sesamum. *Indian Phytopath.*, **10** : 38-47.

POWERS, H. R. AND G. B. LUCAS (1952) Variation in *Phytophthora parasitica* var. *nicotianeae*. *Plant Dis. Reptr.*, **36** : 335-336.

*RIEGER, H. (1935) *Phytophthora* prevention and control (*Phytophthora*—verbenge and Bekampfung) *Dtsch. landw.Pr.* **62** *135 pp.*

TUCKER, C. M. (1931) Taxonomy of the genus *Phytophthora* de Bary *Res. Bull. Missouri agric. Exp. Sta. No. 153.*

UPPAL, B. N. AND N. T. KULKARNI (1937) Studies in *Fusarium* wilt of Sann-hemp I. The physiology and biology of *Fusarium vasinfectum* Atk. *Indian J. agric. Sci.*, **7** : 413-442.

VENKATAKRISHNAIYA N. S. (1950) *Phytophthora parasitica* on French Bean, *Phaseolus vulgaris* L. *Indian J. Agric. Sci.*, **20** : 391-394.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SOME PHYSIOLOGICAL STUDIES ON SUGARCANE SMUT (USTILAGO SCITAMINEA SYD.)*

P. APPALANARASAYYA

(Accepted for publication October 5, 1964)

INTRODUCTION : Of the various diseases of sugarcane prevalent in India, smut is an important disease and is of widespread occurrence throughout the country. During the course of studies at Delhi, it was found difficult to germinate the spores under laboratory conditions.

Luthra *et al.*,[1] studied the germination of sugarcane smut spores in distilled water at temperatures ranging from 5 to 40° C and concluded that the optimum temperature for germination is about 30°C, the maximum between 36 and 40° C and the minimum between 5-13° C. The germinated spores produced sporidia at all temperatures except at 36° C and their number varied from one to three. Rafay and Agarwala[2] studied the germination of smut spores obtained from 26 varieties at temperatures 6, 27, 30, 35° C and room temperature (15 to 25° C). It was found that the spores collected from different varieties do not behave alike in their germination. In a few collections such as those from X 6269, Co. 932, Co. 963 and Co.323, the spores could germinate well even at 6° C (21° C below the optimum).

Luthra *et al.*[1] found that glucose solution at 0.5 to 5.0 per cent was found to be the best for the germination of sugarcane smut spores.

MATERIAL AND METHODS : In the present studies, 1 per cent water agar was employed. Some difficulty was experienced in taking the germination counts in the ordinary methods *viz.*, (*i*) Putting spores on a drop of water kept on a slide and (ii) by hanging drop method. In the first method the spores that are at the periphery of the water drop alone were observed to germinate while the spores at the centre of the water drop did not germinate. In the second method all the spores were observed to cluster at the convex end of the hanging drop.

Smut spores were collected from naturally occurring smut infection on Co. 419 and used throughout the present investigations.

A uniform spore suspension (30 to 40 spores per microscopic field under low power) was prepared and 3 ml. of it was spread on petri-plate containing water-agar medium. Ten such plates were prepared and kept for germination at 10, 15, 20, 25, 30, 35, 45, 50, 55 and 60° C. Germination percentage was determined after 12 hours, as the germination of smut spores under optimum conditions has been found to commence in 3 to 4 hours time and completed within 12 hours.

*Part of thesis approved for the degree of Master of Science by the Council of Post-graduate School at I. A. R. I, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Sucrose, Lactose, Dextrin, Mannite, Mannose and Fructose were used to determine the effect of these sugars on germination at 0.5 percent, 1 percent and 1.5 per cent concentrations. All these treatments were kept at 25° C as this temperature was found optimu n for germination in the studies reported above.

TABLE 1 : Effect of different temperatures on germination of sugarcane smut spores

S. No.	Temperature (°C)	Percent germination.	Remarks
1.	10	Nil	Spores did not germinate even after keeping at this temperature for 96 hours.
2.	15	68	Promycelium branched. Few sporidia formed.
3.	20	74	Promycelium branched. Sporidia formed in large numbers.
4.	25	79	Promycelium long and branched but no sporidia.
5.	30	72	Promycelium branched. No sporidia.
6.	35	65	Promycelium not branched. No sporidia.
7.	45	61	Promycelium not branched. No sporidia.
8.	50	24	Promycelium not branched. No sporidia.
9.	55	2	Promycelium not branched. No sporidia.
10.	60	Nil	—

RESULTS : It will be seen from the above results that maximum germination was observed at 25° C. At temperatures above 25° C germination was recorded upto 55° C but it gradually decreased to 2 per cent at 55° C. Luthra *et al.*, reported that the optimum temperature for the germination of the smut spores is about 30° C, the maximum between 36 and 40° C,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Effect of sugars on germination of sugarcane smut spores

S. No.	Sugars	Percentage of germination at different concentrations		
		0.5 per cent	1.0 per cent	1.5 per cent
1.	Sucrose	80.5	77.3	81.6
2.	Lactose	48.7	57.0	70.1
3.	Dextrin	84.2	81.6	79.0
4.	Mannite	56.7	58.7	63.6
5.	Mannose	71.5	76.0	70.0
6.	Fructose	75.7	75.7	22.5

Control (Water - agar)—60.7 per cent germination

It would be observed from the data that sucrose, dextrin and mannose recorded increased germination over control (water- agar) at all the three concentrations. In case of lactose and mannite there was some increase in germination at 1.5 per cent concentration. Fructose has increased the percentage of germination at 0·5 per cent and 1.0 per cent concentrations, but at 1.5 per cent concentration, the germination was markedly depressed. The marked depression in percentage of germination from 75.7 in 1 per cent fructose to 22.5 in 1.5 percent fructose appears to be due to plasmolysis.

SUMMARY

Optimum temperature for germination of sugarcane smut spores has been determined to be 25° C.

Appreciable increase in germination of the smut spores has been observed with all the sugars except lactose and mannite. In the case of fructose, however, marked depression in germination has been observed at 1.5 per cent concentration.

ACKNOWLEDGEMENTS : I wish to express my deep sense of gratitude to Dr. B. L. Chona, Head of the Division Mycology and Plant Pathology Indian Agricultural Research Institute, New Delhi for his valuable guidance and keen interest during the course of investigations while I was his student for M. Sc., degree.

Grateful thanks are due to the Director of Agriculture Hyderabad, Andhra Pradesh for having deputed me for post-graduate studies at Indian Agric. Res. Institute, New Delhi.

Sugarcane Research Station,
Anakapalle,
Andhra Pradesh.

[1]Luthra, J. C., A. Sattar, and S. S. Sandhu, *Indian J. agric. Sci.*, **8** : 849-861, (1938).

[2]Rafay, S. A. and Agarwala *Proc. IIIrd All India Conf. Sug. Res, and Dev.* workers in India. I. C. S. C. pp. 351-354, (1959).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE NATURE OF RESISTANCE OF PADDY TO HELMINTHOSPORIUM ORYZAE BREDA DE HAAN

A. P. MISHRA AND Y. PRASAD

(Accepted for publication October 1, 1964)

INTRODUCTION : The morphological and physiological bases of resistance of certain varieties of paddy to *Helminthosporium oryzae* Breda de Haan have been studied by many workers. Suzuki (1934) showed a correlation between the resistance of rice leaves to *Ophiobolus miyabeanus* and the extent of silicification of the epidermal cell walls. Chattopadhyaya and Chakrabarti (1957) observed that the susceptible varieties possessed thinner epidermal layer and less number of silicated epidermal buliform cells. Pushpanden (1957) showed increase in ascorbic acid content of plants with age but it was lower in varieties susceptible to leaf spot than in resistant ones. Akai and Asada (1954) and Asada and Akai (1955) observed inhibition of conidial germination and the elongation of their germ tubes in expressed cell sap from *Kameji*, a resistant variety.

The present investigations were undertaken with the object of finding out whether the silicification of epidermal cells of the host, silica content, ascorbic acid content, sugar content and the stomatal movement had any relation to the resistance or susceptibility of a variety of paddy.

MATERIAL AND METHODS : The fungus was isolated from diseased paddy leaves, collected from Sabour in August 1961 and brought into pure culture.

Ten varieties of paddy, *viz.*, AC 2465, SR 26B, AC 2559, AC 1382, AC 2045, Benibhog, AC 1351, CH 45, BAM 10 and BK 36 were included in these studies. These were grown in pots in quadriplicate in randomized blocks and their mean infection percentages were recorded on the basis of the *Helminthosporium* infection Grade Chart of the F.A.O. which has 0 to 9 grades, 0 being immune and 9 being most susceptible. The averages of the readings have been recorded upto two decimal places.

Transverse sections (12 μ thick) were cut with microtome and stained with Safranine and Fast green and permanent mounts made in Canada balsam according to the method described by Johansen (1940).

Spore germination studies were carried out in van Tiegham cells at 30°C. Host extracts were prepared by grinding a weighed quantity of the stems and leaves of paddy in mortar and pestle. The ground material was mixed with $2\frac{1}{2}$ times its weight of sterile distilled water and placed in refrigerator for two hours. The plant juice was extracted by squeezing and filteration. A drop of this juice was placed on a sterile cover slip on van Tiegham rings and spores from 10 to 12 days old PDA cultures were incubated in these for germination.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The stomatal movement at different time intervals was studied by fixing the leaves in absolute alcohol and treating them with pyridine for 24 hours. Leaf pieces (about 1 cm. in diameter) thus treated were mounted in glycerine and examined under the microscope.

The numbers of silicated epidermal cells were estimated by the spodogram method (Warner, 1928). Leaf pieces 5×2 mm. were cut from the sides of the midrib and placed in porcelain dishes. They were then subjected to slow continuous heat over gas flame for 4 to 6 hours, until the leaf bits turned to ash. The material was allowed to cool and the leaf bits removed carefully with camel hair brush, dipped in xylol and mounted on glass slides in pure xylol. The silicated cells in a unit area were counted under the microscope (Adyantha and Rangaswami, 1952).

The sugar content in leaf extracts was determined by the Spencer Refractometer.

The vitamin C (Ascorbic acid) content was determined as follows: A known quantity (12 gm) of leaves and pieces of stem was ground in a measured quantity of distilled water in mortar and pestle and the solution made up to 150 ml. Fifty ml. of this was taken in a conical flask and titrated against N/100 iodine solution using starch as indicator and the neutral point determined. The ascorbic acid content was then calculated according to the quantity of iodine solution used by the following formula.

1 ml. of N/100 iodine=0.88 mg. ascorbic acid.

The silica content was estimated according to the method of Piper (1950).

EXPERIMENTAL: MORPHOLOGICAL RESISTANCE: (i) *Thickness of epidermal wall*: The mean thickness of the epidermal-*cum*-cuticular layer in different paddy varieties was determined. The data are summarised in Table 1.

TABLE 1: Mean thickness of outer epidermal wall-*cum*-cuticular layer of paddy varieties and their mean infection grades

Varieties	Thickness in μ	Infection grades
AC 2045 (V_5)	2.13	3.90
AC 2559 (V_3)	2.36	3.70
AC 1382 (V_4)	2.38	3.60
BK 36 (V_{10})	2.36	3.60
Benibhog (V_6)	2.37	3.50
AC 2465 (V_1)	2.50	3.25
AC 1351 (V_7)	2.97	3.50
SR 26 B (V_2)	3.16	1.20
CH 45 (V_8)	3.15	1.20
BAM 10 (V_9)	3.47	1.10

CD (P=0.05)=0.19

for thickness of epidermal wall-*cum*-cuticular layer

Treatment mean

V_9	V_2	V_8	V_7	V_1	V_4	V_6	V_3	&	V_{10}	V_5
3.47	3.16	3.15	2.97	2.50	2.38	2.37		2.36		2.13

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

There was significant difference in the thickness of epidermal wall cum cuticular layer between different varieties. The varieties having the minimum infection grades possessed the thickest epidermal-*cum*-cuticular layers and *vice versa* (Figs. 1 and 2).

(ii) *Silicated epidermal cells* : The average numbers of silicated epidermal cells of various types in each variety were estimated according to the method described under Material and Methods. The data have been summarised in Table 2.

TABLE 2 : Mean number of silicated epidermal cells in leaves of different paddy varieties

Varieties	Long cells	Short cells	Motor cells	Rice cells	Infection grades
AC 2045	1.70	15.5	10.1	24.3	3.90
AC 2559	2.10	16.1	10.3	30.6	3.70
AC 1382	2.30	16.3	10.7	27.9	3.60
BK 36	3.90	13.9	14.1	24.8	3.60
Benibhog	1.80	16.9	9.5	20.6	3.50
AC 2465	3.60	16.6	11.3	23.9	3.25
AC 1351	4.30	13.2	14.6	23.3	2.30
SR 26 B	9.00	21.4	15.7	35.2	1.20
CH 45	12.30	21.7	25.9	37.3	1.20
BAM 10	14.70	24.9	26.8	41.1	1.10

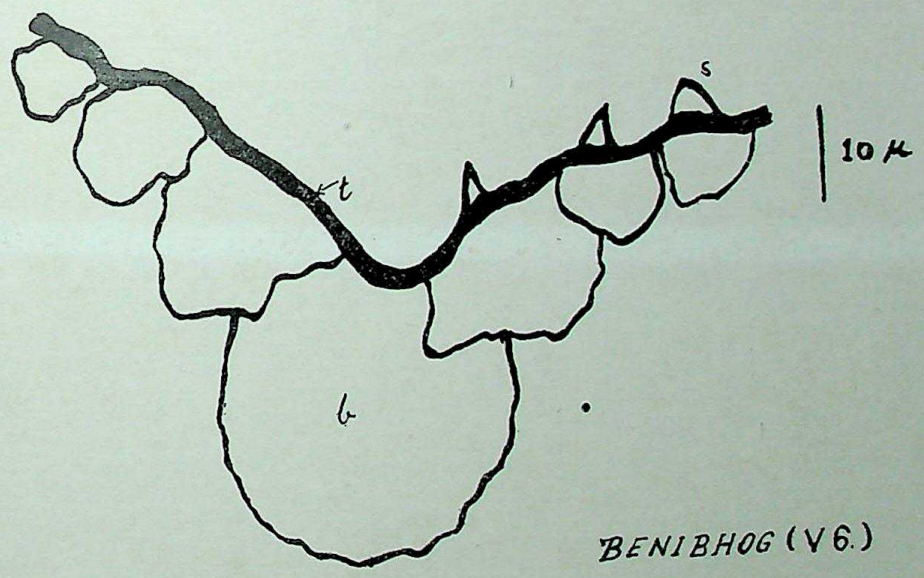


Fig. 1. Camera Lucida Drawings of buliform Cell and epidermal wall cum Cuticular layer in two vars. of paddy

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

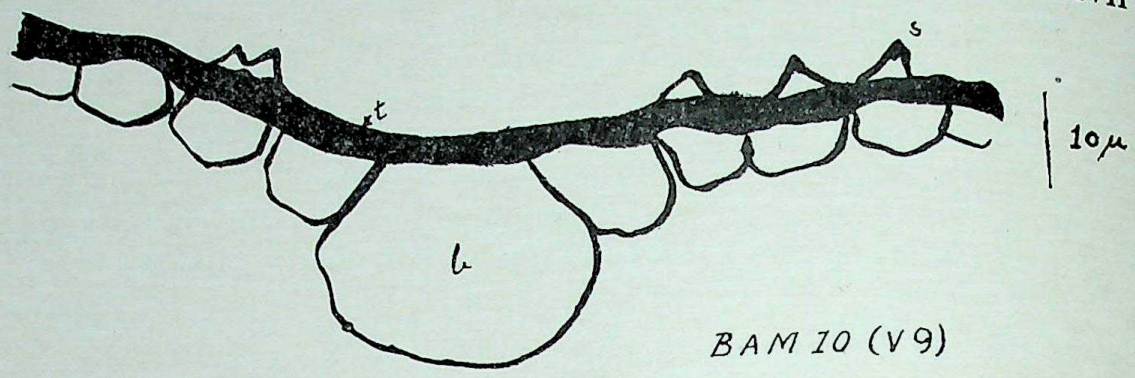


Fig. 2. (b) Buliform cell. t. thickness of epidermal wall cum cuticular layer S. silicification of cells.

There were differences in the numbers of silicated cells between different varieties. The varieties showing low infection grades, *viz.*, SR 26 B, CH 45, and BAM 10 possessed larger number of silicated cells of all types than varieties having higher infection grades.

(iii) *Silica content* : The silica content in different paddy varieties was also estimated but there was no correlation between the total silica content and the infection grades.

(iv) *Stomatal resistance* : (a) *Stomatal number* : The average number of stomata at the apex, middle portion and the base of both the leaf surfaces were counted. Data are summarised in Table 3.

TABLE 3 : Number of stomata on leaves of different paddy varieties

Varieties	No. of Stomata				Infection grades
	Apex	Middle	Base	Mean	
AC 2045 (V_5)	19.75	20.70	20.10	20.18	3.90
AC 2559 (V_3)	24.35	25.25	24.20	24.53	3.70
AC 1382 (V_4)	20.65	21.15	20.65	20.82	3.60
BK 36 (V_{10})	21.75	20.50	21.90	22.05	3.60
Benibhog (V_6)	19.30	20.25	19.55	19.70	3.50
AC 2465 (V_1)	25.15	26.35	25.00	25.50	3.25
AC 1351 (V_7)	19.30	19.85	19.30	19.48	2.30
SR 26 B (V_2)	23.20	24.40	23.25	23.62	1.20
CH 45 (V_8)	18.50	19.35	18.50	18.78	1.20
BAM 10 (V_9)	19.40	20.55	19.10	19.68	1.10
Mean	21.13	22.01	21.15		

Varieties	V_1	V_3	V_2	V_{10}	V_4	V_5	V_6	V_9	V_7	V_8
Treatment mean for no. of stomata	25.50	54.53	23.62	22.05	20.82	20.18	19.70	19.68	19.48	18.78
Infection grades	3.25	3.70	1.20	3.60	3.60	3.90	3.50	1.10	2.30	1.20

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The average number of stomata in different varieties varied significantly, but there was no definite correlation in the number of stomata and the infection grades.

(b) *Stomatal movement* : The movement of stomata in the ten varieties was studied. The data are presented in Table 4.

TABLE 4 : Stomatal condition of different paddy varieties at different hours during the day

Varieties	Stomatal condition during						
	Morning hours				After noon		
	5.15 A.M.	6.15 A.M.	7.15 A.M.	8.15 A.M.	1.15 P.M	6.15 P.M.	Infection grade
AC 2045 (V_5)	F.C.	P.O.	F.O.	F.O.	F.O.	P.O	3.90
AC 2559 (V_3)	P.O.	P.O.	F.O.	F.O.	F.O.	P.O.	3.70
AC 1382 (V_4)	P.O.	P.O.	F.O.	F.O.	F.O.	F.C.	3.60
BK 36 (V_{10})	P.O.	P.O.	F.O.	F.O.	F.O.	P.O.	3.60
Benibhog (V_6)	F.C.	P.O.	F.O.	F.O.	F.O.	P.O.	3.50
AC 2465 (V_1)	P.O.	P.O.	F.O.	F.O.	F.O.	P.O.	3.25
AC 1351 (V_7)	P.O.	P.O.	F.O.	F.O.	F.O.	P.O.	2.30
SR 26 B (V_2)	F.C.	F.C.	P.O.	F.O.	F.O.	P.O.	1.20
CH 45 (V_8)	P.O.	P.O.	P.O.	F.O.	F.O.	F.C.	1.20
BAM 10 (V_9)	P.O.	P.O.	P.O.	F.O.	F.O.	F.C.	1.10

P.O. = Partially open, F.O. = Fully open, F.C. = Fully closed.

The stomata on both the surfaces opened or closed simultaneously but there were differences in the time of opening and closing in different varieties. In the varieties, V_1, V_3, V_4, V_7 and V_{10} the stomata were partially open at 5.15 A.M., whereas those of varieties V_2, V_5, V_6, V_8 and V_9 were fully closed. The stomata of varieties V_2, V_8 and V_9 were only partially open till 7.15 A.M.

In the evening the stomata of varieties V_4, V_8 and V_9 were fully closed at 6.15 P.M. Barring few exceptions the varieties having low infection grades had their stomata open for less time than others, whereas in varieties with higher infection grades, the stomata opened earlier and remained open till late hours in the evening.

Physiological resistance : An experiment was set up to see whether there was any difference in conidial germination and the lengths of germ tubes in extracts from different paddy varieties. The spores were germinated in van Tiegham cells at 30°C. The data are summarised in Table 5.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 5 : Percentage of spore germination of *H. oryzae* and the length of germ tube on extracts from different paddy varieties

Varieties	Percentage of spore germination after			Length of germ tube in μ after		
	12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.
AC 2045 (V_5)	82.38	92.86	94.60	273.80	297.54	410.08
AC 2559 (V_3)	80.44	91.36	94.86	251.02	285.45	297.99
AC 1382 (V_4)	79.36	87.86	93.53	176.36	290.47	371.49
BK 36 (V_{10})	89.50	91.06	95.70	331.90	378.42	395.74
Benibhog (V_6)	88.73	91.46	93.90	270.31	315.57	333.90
AC 2465 (V_1)	81.87	90.70	94.83	291.38	366.10	386.58
AC 1351 (V_7)	75.34	86.96	92.93	194.40	239.44	258.71
SR 26 B (V_2)	72.68	74.43	82.33	126.36	210.21	223.59
CH 45 (V_8)	69.86	79.20	81.93	141.50	190.17	240.16
BAM 10 (V_9)	66.66	73.62	80.70	132.82	155.60	180.06
Control Distilled water.	91.33	94.30	97.40	363.36	402.91	416.51

C.D. (P=0.05)=2.89

Varieties	V_9	V_8	V_2	V_7	V_4	V_6	V_5	V_1	V_3	V_{10}	Control
Per cent spore germination (after 36 hrs.)	80.70	81.93	82.33	92.93	93.53	93.90	94.60	94.83	94.86		97.40
Infection grade	1.10	1.20	1.20	2.30	3.60	3.50	3.90	3.25	3.70		3.60
Mean length of germ tube	V_9	V_2	V_8	V_7	V_3	V_6	V_4	V_1	V_{10}	V_5	Control
(after 36 hours)	180.06	223.59	240.16	258.71	297.79	333.90	371.49	386.58	395.74	410.08	416.51
Infection grade	1.1	1.2	1.2	2.3	3.7	3.5	3.6	3.25	3.60	3.9	

The percentage of spore germination was minimum in extracts of varieties V_9, V_8 and V_2 with low infection grades as compared with other varieties, but among themselves, the differences were non-significant. Similarly the length of germ tubes was also minimum in extracts of these varieties. Thus the extracts of varieties which had minimum infection grades inhibited both the germination percentage of spores and the length of their germ tubes as compared with more susceptible varieties.

Ascorbic acid content : The ascorbic acid content in different varieties at preflowering stage was determined. The results are presented in Table 6.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 6 : The ascorbic acid content in different paddy varieties

Varieties	Ascorbic acid content* mg./100 gm. of plant material.
AC 2045 (V_5)	87.92
AC 2559 (V_3)	92.97
AC 1382 (V_4)	87.13
BK 36 (V_{10})	82.73
Benibhog (V_6)	92.25
AC 2465 (V_1)	90.73
AC 1351 (V_7)	97.67
SR 26 B (V_2)	115.73
CH 45 (V_8)	114.23
BAM 10 (V_9)	119.23

*Average of three replicates.
C.D. (P=0.05)=3.50
Treatment mean

V_9	V_2	V_8	V_7	V_3	V_6	V_1	V_5	V_4	V_{10}
119.23	115.73	114.23	97.67	92.97	92.25	90.73	87.92	87.13	82.73

The ascorbic acid content was maximum in varieties with low infection grades (V_9 and V_2), followed by the variety V_8. In varieties with higher infection grades the ascorbic acid content decreased.

Sugar content : The sugar content of the extracts from different paddy varieties was determined by a Spencer Refractometer. There was a highly significant variation in sugar content in different varieties. The varieties with low infection grades, V_8, V_9 had less sugar content than the varieties V_1, V_3, V_4, V_5, V_6, and V_{10} with higher infection grades (Table 7).

TABLE 7 : Sugar percentage in host extracts from different varieties of paddy

Varieties	V_5	V_3	V_4	V_{10}	V_6	V_1	V_7	V_2	V_8	V_9
Sugar per cent	1.055	0.8867	0.9433	1.00	0.775	0.9433	0.665	0.72	0.50	0.555
Infection grade	3.90	3.70	3.60	3.60	3.50	3.25	2.30	1.20	1.20	1.10

DISCUSSION : In a study of the morphological, physiological and functional bases of resistance in the varieties of paddy (AC 2465, SR 26B, AC 2559, AC 1382, AC 2045, Benibhog, AC 1351, CH 45, BAM 10, BK 36) to *Helminthosporium oryzae*, it was observed that there were significant differences among themselves in respect of the thickness of their epidermal wall cum cuticular layers, number of silicated epidermal cells, sugar and ascorbic acid content of the leaves and the ability of the host extracts to support the germination of spores and the elongation of the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

germ tubes. Differences were also observed in time and duration of opening of their stomata.

The varieties BAM 10, SR 26B and CH 45 had low infection grades, were comparatively resistant, and had thicker epidermal wall cum cuticular layer and larger number of silicated epidermal cells. Akai and Asada (1954) made similar observations in the variety *Kameji* studied by them. It was interesting that there was positive and negative correlation respectively between the number of silicated epidermal cells, *viz.*, the long cells, short cells, motor cells and the rice cells in resistant and susceptible paddy varieties, but no such correlation existed with their total silica content. Evidently the silicated nature of the cells is more important in resisting fungal invasion than the total silica content.

The resistant varieties with low infection grades had a higher ascorbic acid content but the converse was true for the sugar content. Pushpandan (1957) and Gupta (1961) reported somewhat similar result as regards the ascorbic acid content with the varieties studied by them. The results in respect of the sugar content were similar to those of Akai and Asada (1955).

The percentage of spore germination and the length of germ tubes was minimum in extracts of varieties BAM 10, CH 45 and SR 26 B as compared to relatively susceptible varieties. This corroborates the earlier results of Gupta (1961) in respect of varieties studied by him. Akai and Asada (1945) also reported that the expressed cell sap of resistant variety *Kameji* inhibited the elongation of germ tubes.

It was interesting that the time of opening and closing of stomata and the duration they remained open were also different. During the early morning hours and late in the afternoon when the chances of infection are more favourable the stomata of the more resistant varieties were either closed or only partially open.

Though any single factor may not be decisive in determining the resistance or susceptibility of any variety to the disease, their combined effect is likely to matter a great deal and the factors studied do not exhaust the list of other causes that may be operative.

Summary

The morphological and physiological bases of resistance in ten varieties of paddy, *viz.*, AC 2465, SR 26 B, AC 2556, AC 1382 AC 2045, Benibhog, AC 1351, CH 45, BAM 10 and BK 36, differing in their reaction to *Helminthosporium oryzae* were investigated.

The varieties BAM 10, SR 26 B and CH 45, having low infection grades had thicker epidermal cell wall cum cuticular layers and larger number of silicated epidermal cells as compared to varieties with higher infection grades.

The stomata in varieties CH 45 and BAM 10 (with low infection grades) were open for a shorter period than in case of varieties having higher infection grades.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The percentage spore germination and the length of germ tubes were minimum in extracts of varieties BAM 10, CH 45 and SR 26 B compared with extracts of relatively susceptible varieties.

The ascorbic acid content was higher in relatively resistant varieties, but the converse was true for sugar content.

ACKNOWLEDGEMENT : Thanks are due to Sri R. S. Roy, Principal, Bihar Agricultural College, Sabour, for providing necessary facilities for this work.

Department of Plant Pathology,
Bihar Agricultural College,
Sabour, (Bhagalpur)

REFERENCES

ADYANTHA, N. R. AND G. RANGASWAMI (1952) Distribution of silica in relation of resistance to blast disease of rice. *Madras Agri. J.*, **39** : 198-204.

*AKAI, S. AND Y. ASADA (1954) Studies on Helminthosporium blight of rice plants on the resistance of varieties of rice plants to Helminthosporium blight or blast disease in rice plants. *Forsch. Pflkr. Kyoto*, **5** : 14.

*ASADA, Y. AND S. AKAI (1955) Effect of expressed cell sap of rice plant leaves upon the germination of *Cochliobolus miyabeanus* conidia. *Shokubutsu-Byogai-Kenkyu*, **5** : 63-66.

CHATTOPADHYAY, S. B. AND N. K. CHAKRABARTY, (1957) Relationship between anatomical characters of leaf and resistance to infection of *Helminthosporium oryzae* in paddy. *Indian Phytopath.*, **10** : 130-132.

GUPTA, S. F. (1961) *Study on the leaf spot of paddy caused by* Helminthosporium oryzae *Breda de Haan with special reference to the nature of host resistance.* M. Sc. thesis, Bhagalpur University.

JOHANSEN. D. A. (1940) *Plant microtechnique.* McGraw Hill Book Co., Inc., New York and London.

PUSHPANDEN, P. (1957) Ascorbic acid and *Helminthosporium* of *Oryza sativa. Curr. Sci.*, **26** : 26.

PIPER, C. S. (1950) *Soil and plant analysis.* Inter Science Publishers Inc., New York.

*SUZUKI, H. (1934) Studies on the influence of some environmental factors on the susceptibility of rice plants to blast and Helminthosporium diseases and on the anatomical character of plant. I. Influence of differences in soil moisture. *J. Coll. Agric. Tokyo*, **13** : 45-108.

WARNER, C. (1928) *Biologia generalis.* **4** : 403-446.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MUTATION IN COLLETOTRICHUM FALCATUM WENT, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE

IV. MORPHOLOGICAL AND BIOCHEMICAL MUTANTS INDUCED BY IONIZING RADIATION

B. S. Bajaj*, M. S. Chatrath and R. S. Vasudeva

(Accepted for publication October 5, 1964)

It has been observed earlier that *Colletotrichum falcatum* is highly mutable on artificial culture media. (Lucas, 1942; Ramakrishnan, 1942; Chona and Hingorani, 1950). The frequency and spectrum of mutation in this fungus are reported to be markedly increased by irradiation and by treatment with radiomimetic chemicals like nitrogen mustard (Vasudeva *et al.*, 1957; Bajaj and Chatrath, 1958; Bajaj and Dhanraj, 1960), resulting into pronounced morphological changes in shape and size of spores, colour of the colony and spore pigments (Vasudeva *et al.*, 1958; Bajaj, Ganju and Chatrath, 1959; Bajaj and Chatrath, 1960). No studies appear to have so far been made on physiological variation in this fungus. Attempts have been made to induce mutation in biochemical characters of the red rot pathogen, with the aid of ionizing radiation. Attempts were also made to study the spectrum of morphological mutation, especially in substrate pigmentation, colony characteristics and nature of sporulation. The results obtained are presented here.

Material and Methods : The wild strain used in these studies was a light type highly sporulating virulent isolate of *C. falcatum* (No. 244) obtained from the Indian Type Culture Collection and was purified by single spore isolation at the beginning and used as parent culture throughout these investigations.

Different types of radiation sources were employed as mutagenic agents for these studies. Gamma-irradiation facility was provided by the United States Atomic Energy Commission through a Cobalt^{60} source installed at the World Agricultural Fair at New Delhi (Brewbaker and Swaminathan, 1960) and by the Tata Memorial Hospital, Bombay through a source of Radon (Rn) gas (Vasudeva *et al.*, 1959). X-ray irradiation facility was also provided by the Tata Memorial Hospital, Bombay through 220 KV-15 mA machine. Neutron irradiation was made through cascade generator, at the Tata Institute of Fundamental Research Bombay (Bajaj, Ganju and Chatrath, 1959). Carrier-free radioactive isotope of Phosphorus^{32} obtained from Atomic Energy Establishment, Trombay in the form of $H_3P^{32}O_3$ or $KH_2P^{32}O_4$, was used as an internal beta irradiation source. The procedure followed for irradiation, and screening of morphological and biochemical mutants was essentially the same as described earlier (Bajaj and Chatrath, 1960). Analysis of deficiency was made using the usual method (Beadle and Tatum, 1945) by incorporating yeast extract (0.1 per cent), Casein hydrolysate (0.5 per cent), nucleic acid (DNA) (0.025 per cent). separately and in mixture into the

*Present address : Deputy Superintendent, Antibiotic Project, Virbhadra, Rishikesh (U. P.).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

minimal medium (DNP*) so as to develop an initial selective medium. Further analysis of deficiency of vitamin was made by incorporating different vitamins into the minimal medium, separately and in mixture. The vitamins and their concentrations used in these studies were same as reported by Chatrath and Bajaj (1964).

In all the experiments relating to deficiency analysis, the inoculum, whether spore or mycelial, was washed twice by centrifugation and inoculations made by the "spot inoculation method" where the spores were incorporated either in Bacto Plain agar or in the minimal medium and by means of a loop of the inoculating needle a drop deposited in the centre of the slant so that it set immediately to form an inoculated spot. This method was somewhat similar to the 'point inoculation method' adopted by Bacon, Burrows and Yates (1950) for isolating biochemical mutants of *Bacterium typhosum*. Heavy concentration of the inoculum was used in all the experiments. Absence of growth around inoculation spot on slant culture after 10-day incubation at 25°-27° C was taken as criterion for deficiency. Growth covering $\frac{1}{4}$ to $\frac{1}{2}$ of the slant length was taken as indication of partial deficiency. Changes in colour, texture and nature of mycelium; colour, nature and intensity of spore masses and microscopic characters in comparison with unirradiated cultures were considered as criteria for morphological variation.

Acid washed Pyrex-glassware and analytical grade chemicals were used throughout the investigation relating to deficiency analysis. Bacto agar (Difco) was incorporated (2 per cent) in the medium for the preparation of solid media. The media were sterilised at 10 lb. p. s. i. pressure for 10 minutes. All the cultures were maintained on oat meal agar slants.

EXPERIMENTAL : *Morphological variation* : For the study of morphological variation in *C. falcatum*, the culture of isolate No. 244 was repeatedly irradiated in growth phase as also by spore treatment using different sources of ionizing radiation and plated on oatmeal agar. Single spore colonies were transferred again on oat meal agar and the progeny of each studied for changes in morphological characters. The sources of radiation, dosages, method of irradiation and population of variants are given in Table 1.

Marked variation in colony characteristics such as change from light to dark type having more of cottony mycelium; production of yellow pigment in the substrate or in the mycelium; and in the type of spore masses, was observed. Most of the morphological changes were induced in experiments where P^{32} was used as internal mutagenic source.

Biochemical variation : In order to induce biochemical mutants of *Colletotrichum falcatum* pure culture of isolate No. 244 was irradiated in growth phase with beta particles by incorporating P^{32} in oatmeal agar. Attempt to induce mutation was repeated 11 times using dosages varying from 100 to 400 microcuries/ml. of P^{32} and period of irradiation from 25 to 62 days. The growth and sporulation in the irradiated cultures were retarded in comparison with the unirradiated cultures of the wild type

*Per litre, Dextrose 30 g; KNO_3 1.33 g.; Mg SO_4. 7 H_2O 0.5 g.; M/30 Sorensen's phosphate buffer at pH 6.0.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar.

TABLE 1 : Population of morphological mutants of *C. falcatum* induced by ionizing radiation

Source and kind of radiation	Dose	Method of irradiation	Period of irradiation	Total number of colonies picked up	No. of morphological mutants				
					Yellow pigmented	Dark mycelial		Light sporulating, masses small	Intermediate type
						Normal spores	Change in spore size & shape		
P^{32} (β)	100 μc/ml.	Growth phase	25 days	449	6	5	0	1	4
P^{32} (β)	241 μc/ml.	-do-	30 days	50	3	1	2	7	0
P^{32} (β)	300 μc/ml.	-do.	58 days	424	1	135	15	2	111
P^{32} (β)	396 μc/ml.	-do-	35 days	100	3	3	0	0	1
Rn (γ)	3,750 r	Spore treatment	34 hrs.	50	0	3	0	3	0
Co^{60} (γ)	75,000 r	Repeated Growth Phase	25 min.	200	45	0	0	0	90
X-rays	12,000 r	Spore treatment	5 min.	50	0	0	0	0	1
Cascade generator (fast neutrons)	$0{\cdot}35 \times 10^7$ Nf/cm.^{2}/Sec.	-do-	2 hours	200	0	0	0	0	0

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(control) and this may be due to the deleterious effect of radiation. In the case of gamma irradiation, Cobalt^{60} source (2111.1 curies) emitting 185, 150 roentgens per hour was employed and growing cultures were irradiated at periodic intervals of 0,72 and 144 hours, after inoculations and cultures incubated for further growth and sporulation.

The spores of irradiated cultures were harvested, washed repeatedly by centrifugation in order to reduce level of activity as far as possible and were plated on oatmeal agar. Further screening of mutants was made as already described. The results of all the 12 experiments together with the source and dose of radiation are summarised in Table 2.

TABLE 2 : Population of biochemical mutants of *C. falcatum* induced by ionizing radiation.

Experiment (Title)	Source and kind of radiation	Dose	Period of irradiation	Total number of colonies picked up	Number of biochemical mutants showing	
					Negligible growth on DNP	Slow growth on DNP
A	P^{32} (β)	390 μc/ml.	51 days	50	2	0
B	P^{32} (β)	300 μc/ml.	45 days	125	2	2
C	P^{32} (β)	100 μc/ml.	33 days	200	0	1
G	Co^{60}(γ)	75,000 r	25 minutes	200	1	28
L	P^{32} (β)	250 μc/ml.	35 days	250	4	0
M	P^{32} (β)	400 μc/ml.	42 days	200	12	11
N	P^{32} (β)	300 μc/ml.	62 days	150	3	3
U	P^{32} (β)	272 μc/ml.	38 days	252	3	25
V	P^{32} (β)	300 μc/ml.	58 days	135	3	32
W	P^{32} (β)	100 μc/ml.	25 days	130	2	1
X	P^{32} (β)	241 μc/ml.	30 days	50	1	0
Y	P^{32} (β)	181 μc/ml.	38 days	107	3	0

In all the experiments, unirradiated controls were maintained under similar conditions and 50 to 100 single spore colonies were picked up along with the irradiated ones. All the control colonies showed normal growth and sporulation on the minimal medium within 6 to 7 days indicating the prototrophic nature of the wild type.

All those cultures which showed restricted or negligible growth on the minimal medium consistently for 2 to 3 conidial generations were selected for further analysis of deficiency. These cultures showed quite a wide range of variability in their morphological characters which included typical dark type with sparse sporulation to typical light type with pink

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spore masses. Some cultures produced yellow pigment in the medium (oat meal agar), particularly in the initial stages of their growth. Barring a few, most of these cultures could grow normally on oat meal agar.

For analysis of deficiency, the selected cultures were tested on initial selective medium, and only those cultures which showed growth on yeast extract were inoculated on the final selective medium, i.e DNP containing optimum dose of different vitamins separately as also in mixture in culture slants, for analysis of vitamin deficiency. The result of analysis based on 10-days growth in respect of 27 mutants are presented in Fig. 1. The population of light and dark type strains with their deficiency is also shown in the figure.

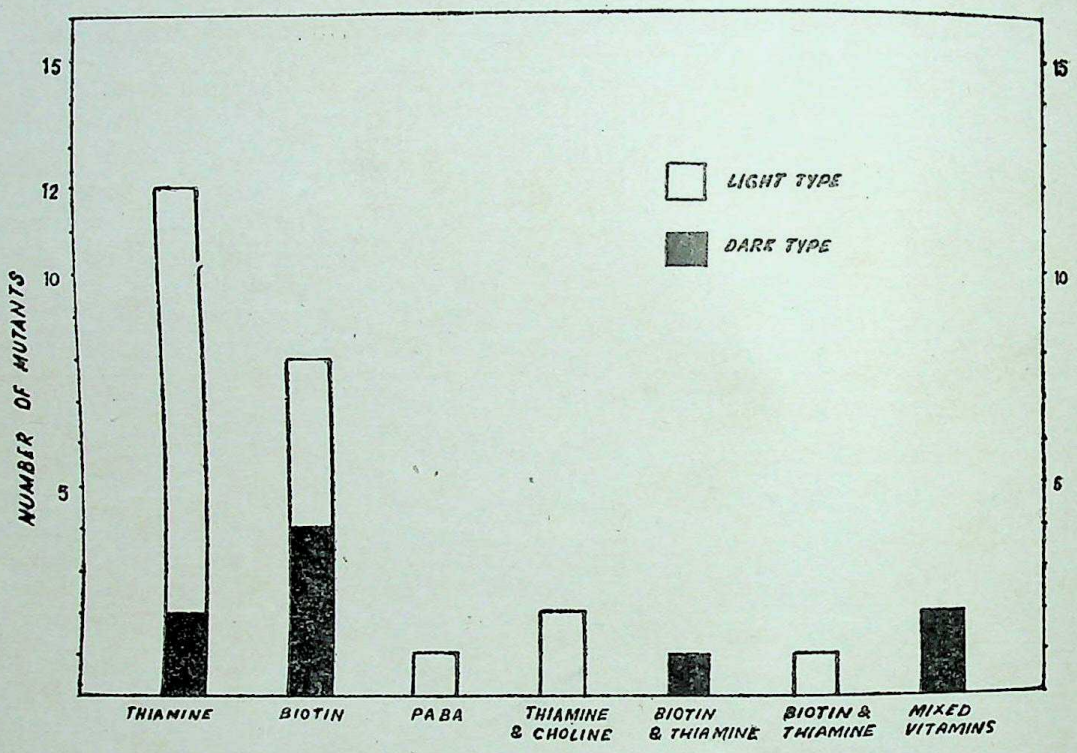


Fig. 1. Spectrum of biochemical mutation in *Colletotrichum falcatum* induced as a result of irradiation with P^{32} (Eleven experiments)

As is evident from the analysis data, the population of thiamine mutants excelled all other deficiencies.

DISCUSSION : Studies on induced mutation in *Colletotrichum falcatum*, the red rot organism of sugarcane, with different sources of ionizing radiation have yielded variety of morphological and biochemical mutants. Though it was difficult to make comparative evaluation of mutagenic efficiency of different sources of radiation tried, particularly because of different dosages applied and different energies and mode of action involved, yet radioactive phosphorus when used as internal mutagen, in general, may be considered more effective than external sources of ionizing radiation tried. Irradiation of cultures with gamma rays from Co^{60}, even upto 75,000 roentgen in growth phase did not yield appreciable results except that number of yellow pigmented variants was comparatively high.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Since no means were available by which inheritance of induced characters could be ascertained more definitely because the wild type strain (No. 244) normally does not form the ascigerous stage, consistency of the induced characters through repeated mass transfers and single spore isolations and stability of the wild type over a period of 17 years suggest that variability reported here is due to induced mutation involving permanent genetic alterations. As the studies were initiated with monoconidial cultures and conidia being uninucleate (Singh and Chona, 1964) and haploid, the possibility of heterokaryosis and parasexuality as factors in the variability obtained in the present studies is ruled out.

Several dosages of P^{32} were tried in growth phase to induce biochemical mutation in *C. falcatum* in different experiments and these yielded mutants deficient in p-aminobenzoic acid, biotin, thiamine. biotin and choline, thiamine and choline, biotin and thiamine and mixed vitamin deficiency showing thereby that organism under study is highly mutation sensitive to β-particles. The biochemical mutants also showed wide range of morphological variability. No correlation, however, appears to exist between the nature of deficiency and morphological character as the mutant deficient in biotin or thiamine were either light type, resembling the wild strain or typical dark type. Although, the range of induced variability in respect of biochemical mutation was not as wide as reported in *Venturia inaequalis* as a result of treatment with nitrogen mustard and ultra violet light (Boone and Keitt 1952; Keitt and Boone 1954; Boone *et al.* 1956) or in *C. lagenarium* as a result of ultra violet irradiation (Datta, Hall and Heyne 1960), yet isolations of certain mutants which did not show any growth on individual vitamins indicated the existence of greater diversity in respect of nutritional deficiency than detected in the above experiments. Furthermore, these studies were confined only to vitamin deficiency. The biochemical mutant of *C. falcatum*, deficient in p-aminobenzoic acid, was considered interesting, particularly because such mutants do not appear to have been reported in plant pathogenic fungi.

SUMMARY

Using different kinds of ionizing radiations, e. g. beta particles (P^{32}), gamma rays (Co^{60} and radon gas), X-rays and fast neutrons, a number of morphological. cultural and biochemical mutants of *Colletotrichum falcatum* Went (isolate No. 244), the causal organism of red rot of sugarcane have been induced and these show wide range of variability, Biochemical mutants, mostly induced by means of P^{32} irradiation in growth phase, showed deficiency for p-aminobenzoic acid, biotin, thiamine, biotin and choline, thiamine and choline and mixture of vitamins. There was, however, no correlation between nature of deficiency and morphological characters.

ACKNOWLEDGEMENTS : The authors are thankful to Dr. B. L. Chona, Head of the Division for going through the manuscript and valuable suggestions. Thanks are also due to U. S. Atomic Energy Commission, Tata Memorial Hospital, Bombay and Tata Institute of Fundamental Research, Bombay for providing irradiation facilities.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Assistance rendered by Shri P. L. Ganju in conducting certain initial experiments is also gratefully acknowledged.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

BACON, G. A., T. W. BURROWS AND MARGARET YATES (1950) The effect of biochemical mutation on the virulence of *Bacterium typhosum*: The induction and isolation of mutants. *Brit. J. Exptl. Pathol.*, **31** 703—713.

BAJAJ, B. S. AND M. S. CHATRATH(1958) Some useful applications of ionizing radiations in Plant Pathological Research *Proc. Mycol. Res. Wkrs. Conf.*, Simla, 61—68. Indian Counc. Agric. Res., New Delhi.

BAJAJ, B. S. AND M. S. CHATRATH (1960) Growth phase method for mutant-screening in *Colletotrichum falcatum* Went. *Indian Phytopath.*, **13** : 156—160.

BAJAJ, B. S. AND K. S. DHANRAJ (1960) Mutation in *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane. III. Some biological effects of Methyl Bis. (β-chloro ethyl) amine. *Indian Phytopath.*, **13** : 48—54.

BAJAJ, B. S., P.L. GANJU AND M.S. CHATRATH(1959) Mutation in *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane. II. induced by fast neutrons. *Indian Phytopath.*, **12** : 53—58.

BEADLE, G. W. AND E. L. TATUM (1945). *Neurospora* III. Methods of producing and detecting mutations concerned with nutritional requirements. *Amer. J. Bot.*, **32** : 678—686.

BOONE, D. M.. AND G. W. KEITT (1952) Induced biochemical mutants in *Venturia inaequalis* (Abs.) *Phytopathology.*, **42** : 479.

BOONE, D. M., J. F. STAUFFER, M. A. STAHMANN AND G.W. KEITT (1956) *Venturia inaequalis* (Cke.) Wint. VII. Induction of mutants for studies on genetics, nutrition, and pathogenicity, *Amer. J. Bot.*, **43** : 199—204

BREWBAKER, J. L. AND M. S. SWAMINATHAN (1960). The design and use of Co^{60} irradiation unit in the United States Exhibit, World Agricultural Fair, *Curr. Sci.*, **29** : 298—301.

CHATRATH, M. S. AND B. S. BAJAJ (1964). Studies on the physiology and pathogenicity of an avirulent strain of *Colletotrichum falcatum*, *Indian J. Sugarcane Res. Dev.*, **8** : 288—292,

CHONA, B. L. AND M. K. HINGORANI (1950). Mutation in *Colletotrichum falcatum* Went, the causal organism of sugarcane red rot. *Phytopathology*, **40** : 221—227.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

DUTTA, S. K., C. V. HALL AND E. G. HEYNE (1960) Pathogenicity of biochemical mutants of *Colletotrichum lagenarium*. *Bot. Gaz.*, **122** : 166—170.

KEITT, G. W. AND D. M. BOONE (1954) Induction and inheritance of mutant characters in *Venturia inaequalis* in relation to its pathogenicity. *Phytopathology.*, **44** : 362—370.

LUCAS, G. B. (1942) Deterioration of red rot fungus in culture. *Proc. La. Acad. Sci.*, **6** : 46—47 (*Rev. appl. Mycol.*, **21** : 303).

PRITAM, SINGH AND B. L. CHONA (1964) Nuclear condition in *Colletotrichum falcatum* Went. *Indian J. Expt. Biol.* **2** : 241-243.

RAMAKRISHNAN, T. S. (1942). Studies in the genus *Colletotrichum*. II. Physiological studies on *Colletotrichum falcatum*. *Proc. Indian Acad. Sci.*, **14** (B) : 395—411.

VASUDEVA, R. S., B. S. BAJAJ, M. R. S. IYENGAR, M. S. CHATRATH AND P. L. GANJU (1957). Mutagenic and stimulative effects of ionizing radiations on certain microorganisms. *Proc. 4th internat. Congr. crop. Prot.*, Vol., **1** : 121—123, Hamburg.

VASUDEVA, R. S., B. S. BAJAJ, M. S. CHATRATH AND P. L. GANJU (1959) Stimulating effect of ionizing radiation on certain micro-organisms *Indian Phytopath.*, **12** : 19—24.

VASUDEVA, R. S., M. R. S. IYENGAR, B. S. BAJAJ AND M. S. CHATRATH (1958) Mutation in *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane. I. Induced by Beta radiation. *Indian Phytopath.*, **11** : 91—95.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A NEW PSEUDOTORULA FROM RAJASTHAN

P. N. MATHUR AND G. SINGH

(Accepted for publication October 10, 1964)

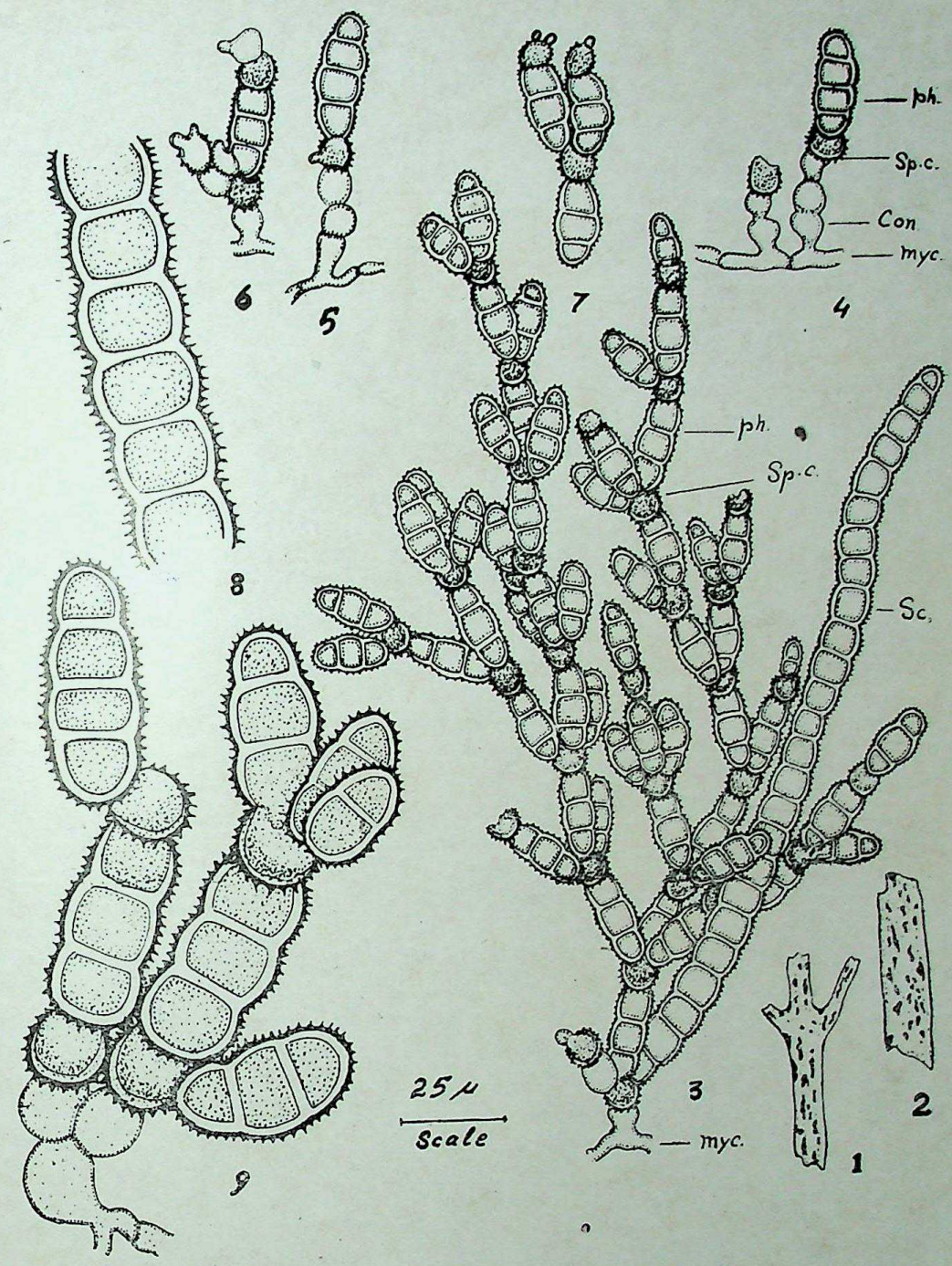
A dark sooty black fungus was found growing on the drying stems and branches of 'Bhindi' (*Hibiscus abelmoschus)* at Budha Pushkar, Ajmer (Figs. 1 and 2). Microscopic examination indicated that this organism belongs to the genus *Pseudotorula* described by Subramanian[1] from Nilgiris. The present fungus differs markedly from *P. heterospora* Subram., probably the only species of the genus known so far. It is, therefore, proposed to describe this as a new member of the genus *Pseudotorula*.

The fungal mycelium consists of fairly stout (5.5μ) pale brown, septate, constricted at septa, and profusely branched hyphae. Many short lateral fertile branches are produced, which pierce the overlying epidermal tissue and project a little above it. These are usually 2 to 3 celled long and are torulose (Figs. 4-6). At the point of contact with the parent hyphae the basal cells of these conidiophores are very narrow and may, therefore, appear to be elongate-clavate in shape (Fig. 4). The basal cell measures 4 - 10μ in length and 4 - 5.5μ in thickness. The other cells of the conidiophores are more or less globose to subglobose, measuring 4 - 5.5μ in diameter, with relatively darker and thicker walls. The terminal cell of a conidiophore is sporogenous (*cf.* Subramanian[1]) in function (Fig. 5-6), The wall in the lower half of this cell is thick, verrucose and darker in colour than the upper half where it is thin, hyaline and smooth (Fig. 9). This unequal development of the wall imparts a cupulate appearance to the sporogenous cell. These cells measure 4 - 6μ in diameter. Sometimes all the cells of a conidiophore may have verrucose walls. A conidiophore may exhibit branching (Fig. 4); two rows of cells may arise from a single basal cell, each terminating in a cupulate sporogenous cell. Occasionally, a conidiophore may proliferate and fresh rows of cells may arise from an old sporogenous cell after the spores or spore chains produced by it have got detached. From the sporogenous cell two kinds of spores are produced - (1) phragmospores, which are produced in profusely branched chains and (2) scolecospores. which are long, unbranched and light brown in colour; only a few of these are produced in a colony.

The phragmospores are small, oval, obovate to oblong, dark brown, 1 to 4 - (mostly 3 - , rarely more) septate and almost invariably constricted at each septum and with thick verrucose walls, measuring 8.5 - 18.5μ in length and 5.6μ in width ; the cell immediately above the lowermost cell is usually the widest (Fig. 9). After attaining a certain size, a phragmospore destined to serve as the starting point for a branch in the phragmospore chain, produces a small sporogenous cell from its uppermost cell. This sporogenous cell is similar in appearance to the one produced at the tip of a conidiophore and can be readily distinguished from the rest of the cells by its more or less cupulate appearance (Fig. 9). One to three or more small pouch-like protuberances, similar to those on the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Pseudotorula verrucospora on H. *abelmoschus*. (Plate 1-9.) Figs. 1 & 2 : Dark black sooty growth on the stem; Fig. 3 : Conidiophore, phragmospores, and scolecospore cells; Figs. 4-7 : Conidiophore with sporulating sporogenous cells. Fig. 8 : Scolecospore cells magnified; Fig. 9 : phragmospore and sporogenous cells, magnified. (Con. conidiophore; Myc. mycelium; Ph. phragmospore cell; Sc. scolecospore cell.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sporogenous cell of a conidiophore, develop from the hyaline upper half of these sporogenous cells, initiating the development of more phragmospores or their chains (Fig. 3). Development of such sporogenous cells during successive generations and the production of new spores or their chains from them results in the development of a profusely branched, more or less bushy, fertile structures. Such cells have not been found developing on the scolecospores.

Ordinarily, the sporogenous cells of a conidiophore may produce a single scolecospore also. Sometimes a scolecospore may be produced by the sporogenous cell developing at the apices of some phragmospores. The scolecospores are long, vermiform, straight or curved and many-celled (Fig. 3). They are pale-brown to light brown, and have verrucose to spiny walls and well marked constrictions at each septum. The cells of their lower regions are often darker in colour than those of the upper regions. Also, the cells at the two extremities of the spores may be smaller in size than those in the middle portions. These spores differ markedly from the phragmospores in being very long, in being lighter in colour and in having relatively less thickened walls, and have relatively longer spiny projections. The lowermost cell of a spore has a well marked scar which represents the point of attachment. The scolecospores measure 35 - 175 × 4 - 5.5μ. The longest spore observed was 32 cells long. The individual cells may measure 4 - 5.5 × 4 - 5.5μ. The apical cell of the spore is rounded at its free end.

The present fungus differs from the type species, *Pseudotorula heterospora* Subram. (Subramanian[2]) in having verrucose phragmospores, in the absence of the development of new phragmospores or their chains right from the apical cell of the lower phragmospores, in their development from a specialized sporogenous cell produced at the tips of the lower spores, in the occasional branching of the conidiophores, in the occasional verrucose appearance of all the cells of a conidiophore, and in the general appearance of the scolecospores, the cells of which are wider than long and have prominently spiny walls. It is considered necessary to create a new species and the name *verrucospora* is proposed for it.

Pseudotorula verrucospora Mathur and G. Singh sp. nov.

Hyphae robustae, septatae, pallide brunneae, ad septa constrictaeet furcatae. Conidiophori breves, erecti, desinentes in cellulas sporogenas nonnumquam verrucosas. Cellulae sporogenae parietibus crassis verrucosis in dimidio inferiori, in superiore vero parietibus gracilibus ornatae, producentes phragmosporum catenas et unam scolecosporam. Phragmosporae minutae, semel ad ter, rare pluries, septatae, constrictionibus ad septa, parietibus crassis verrucosis, magnit. 8.5 - 18.5 × 5 - 6μ. Cellula terminalis phragmosporae producit cellulam specialem sporogenam aspectu similem cellulae sporogenae quae conidiophorum coronat atque etiam producit plures sporas vel sporarum catenas ex dimidio superiori tenuibus parietibus praedito, magnitudine minores cellulis phragmosporarum. Scolecosporae rarissimae in singulis coloniis, longae, vermiculares, constrictione distincta ad septum, parietibus eminenter spinosis pallide brunneis, cellula basali ornata cicatrice bene distincta, magnit. 35 - 175 × 4 - 5.5μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Hyphae stout, septate, light brown, constricted at the septum and branched ; Conidiophores short, erect, terminated by cupulate sporogenous cells. Phragmospores small, 1-3 (or rarely more -) - septate, with constrictions at the septa, and with thick verrucose walls and measuring 8.5 - 18.5 × 5 - 6μ. New spores or spore-chains initiated by the development of small, specialized, cupulate sporogenous cell at the tip of a phragmospore. Scolecospores very few per colony, wormiform (caterpillar-like), 35 - 175 × 4 - 5.5μ, constricted at the septa, spore wall light brown, prominently spiny, basal cell of the spore with a scar.

On *Hibiscus abelmoschus*. Budha Pushkar, Ajmer (Rajasthan) ; Govind Singh, Oct. 1961. Type material deposited in I. A. R. I., New Delhi and at C.M.I., Kew.

The authors are grateful to Rev. Father H. Santapau for the latin translation and to the Head of the Department of Botany and the Principal, Government College, Ajmer for providing facilities for work.

Department of Botany,
Government College, Ajmer.

[1]Subramanian, C. V., *J. Indian Bot. Soc.*, 37 : 57-61, (1958).
[2]Subramanian. C. V., *J. Indian Bot. Soc.*, 33 : 33-34, (1954).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON "UDBATTA" DISEASE OF RICE

N. N. MOHANTY

(Accepted for publication October 18, 1964)

INTRODUCTION : Udbatta disease (*Ephelis oryzae* Sydow-*Balansia oryzae* Narasimhan and Thirumalachar) occurs in the hilly regions (elevation of 1500 to 3500′ a. s. l.) of Koraput, Kalahandi, Boudh-Phulbani and Ganjam districts of Orissa. In Koraput district, the disease is severe on certain local varieties such as 'Assamchuri', 'Hatipanjar' and 'Matimami' and also on some improved varieties like J_1, J_3, J_5, HYB. 5, EF. 86, CH. 62, T. 90 and GEB. 24, etc. Usually 2-3 per cent of earhead infection is observed, but in years of severe incidence, loss upto 10-11 per cent is common in susceptible varieties. Tai and Siang (1948) recorded 5-30 per cent earhead infection in damp fields in the Kuming Lake area of China. Kamat and Patel (1951) reported that 9-11 per cent of the plants were infected in north Kanara district of Bombay State.

The disease is characterised by the emergence of an erect greyish white cylindrical axis much like 'Agarbatti' (incense rod) from the boot leaf sheath instead of a normal earhead and no grain is formed (Fig. 1). The disease is usually noticed after emergence of earhead, but in some cases it can also be recognised from such symptoms as twisting of flag leaf at junctura and a lustrous greyish white coating over the first, second or the third leaf blades alongwith corresponding leaf sheaths from the top.

Experiments and Results : Investigations were undertaken to work out the mode of infection and control. All the field experiments were conducted during 1957-58 to 1962-63 either at Jeypore and Pottangi except the first experiment under mode of infection, discussed below which was perfomed during 1957-58 at Bhubaneswar Farm.

A. Experiments on mode of infection : *Experiment No. 1* : For determining whether the disease is seed-borne, seeds of a susceptible variety J_1 obtained from Jeypore from the crop of 1956-57 that had severe infection of Udbatta disease as well as from Bhubaneswar from the crop of 1956-57 (that had no infection of the disease) were treated with Agrosan GN, and sown separately in pots containing sterilized soil at Bhubaneswar Farm during 1957-58. After flowering, observations on clump infection were recorded by counting the total number of affected and unaffected clumps in each group. There was 2 per cent clump infection in the plants raised from the seeds obtained from Jeypore, whereas, there was absolutely no infection in the plants raised from seeds obtained locally from Bhubaneswar. This indicates that the disease is seed-borne.

Experiment No. 2 : To determine whether the disease is soil borne or not, a field experiment with six treatments were conducted. Seeds of a susceptible variety J_1 obtained from Jeypore were used for the purpose. At the time of sowing, six nursery beds were prepared out of which the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

soils of four nursery beds were drenched with 1 per cent formalin at 2.5 gallons per 10 sq. ft. whereas, soils of the other two nursery beds were not drenched with formalin. In two of the four formalin-drenched nursery beds, soil artificially infested with the pathogen was added by burying 500 affected earheads containing viable pathogen in November of the previous year in soil of an enclosed area of 10 sq. ft. and by turning the latter from time to time during the year to mix the pathogen in the soil thoroughly and in one, untreated seeds were sown. In another, hot water-treated (54° C for 10 minutes) seeds were sown. Of the two nursery beds drenched with formalin alone, in one untreated seeds were sown and in another hot water treated seeds were sown. Out of the two untreated nursery beds, in one untreated seeds and in another

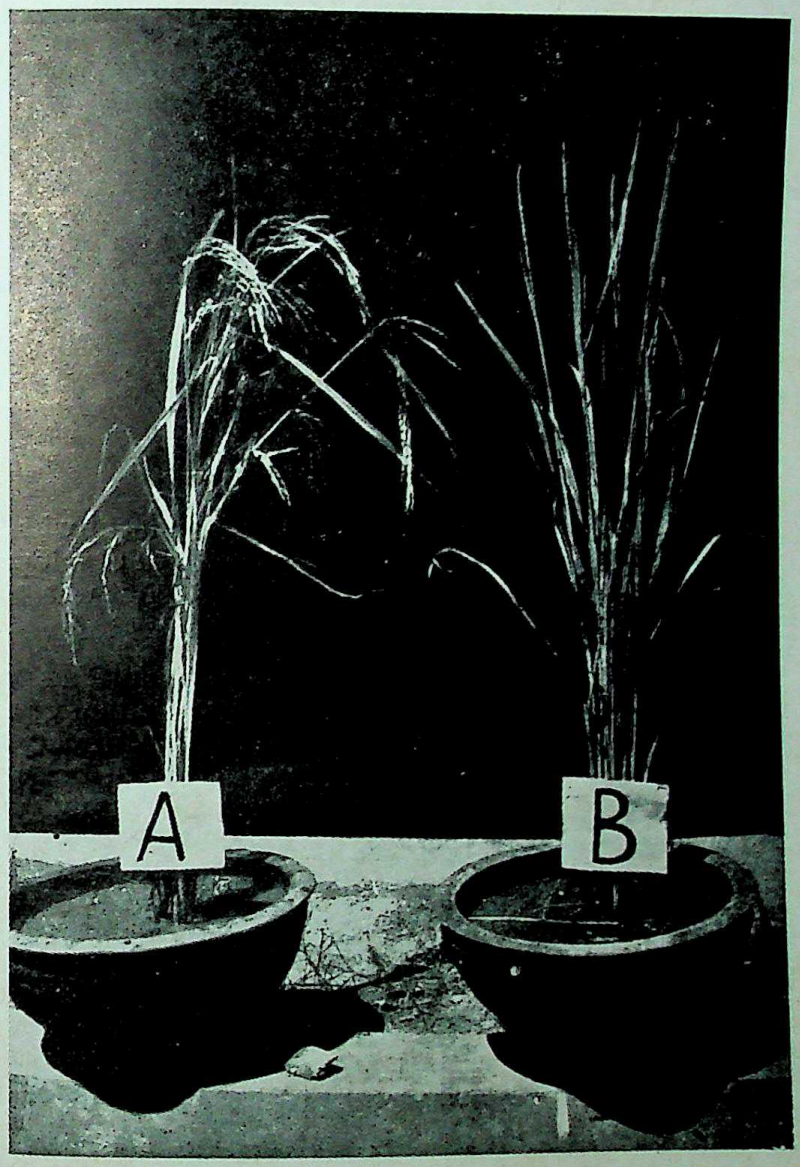


Fig. 1. A. Healthy Plant having normal earheads.
B. Infected Plant having diseased earheads.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

hot water treated seeds were sown. One month old seedlings were transplanted in the field replicated three times in a compact randomized block. After flowering of the plants, observations on incidence of the disease were recorded by counting the total number of affected and unaffected clumps and earheads in each treatment and the data are presented in Table 1.

TABLE 1 : Incidence of Udbatta Disease in different soil treatments (1959-60 and 1961-62)

Treatments	Average percent of clump infection	Average percent of earhead infection
Untreated seeds :		
1. Treated soil	6.88	2.18
2. Treated soil & infested soil	6.50	2.44
3. Untreated soil	4.50	1.37
Hot water treated seeds :		
1. Treated soil	0.22	0.88
2. Treated soil & infested soil	0.27	0.05
3. Untreated soil	0.30	0.04
C. D. (0.05)	4.37	2.06

It may be seen that the effect of the soil treatment with formalin or soil infestation is not reflected in the infection which developed ; the only significant difference in this respect being between hot water-treated seed and untreated seeds.

Experiment No. 3 : To find out whether infection was occurring at the time of flowering or at the time of germination of the seesds in the soil, an experiment was carried out. Seeds of J_1 variety were soaked in ordinary water for 5 hours and then treated with hot water at 54° C for 10 minutes to eliminate seed-borne infection. The hot water treated-seeds were divided into four lots and were germinated separately. The germinated seeds of first lot were smeared with spore suspension of the pathogen and left for 32 hours for enabling the pathogen to infect the germinated seeds and then sown in the nursery bed. The germinated seeds of the 2nd, 3rd and 4th lots were kept for 32 hours and then sown in the nursery beds separately. The nursery beds were bagged with muslin cloth bags to avoid aerial infection. When the seedlings were about one month old, they were transplanted in the field, replicated twice in a compact randomized block.

Immediately after transplanting, the plants of all the treatments were also bagged with muslin cloth bags to avoid aerial infection. Plants of the 2nd and 3rd treatments were inoculated with spore suspension by

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spraying with an atomizer just at the time of emergence of earheads from the boot leaf sheaths and at post-flowering stage respectively. Plants of the 4th treatment were left uninoculated to serve as control. Before harvest of the crop, observations were recorded on disease incidence in each treatment. The data obtained are presented in Table 2. At the time of harvest, seeds were collected from different treatments and stored separately.

In the succeeding year, seeds of different treatments were sown separately and when the seedlings were one month old, they were transplanted in the field in a compact randomized block replicated four times. Plants of all the treatments were bagged from the beginning. Neither seeds nor the plants were inoculated. After flowering, disease incidence was recorded and data are presented in Table 2.

TABLE 2 : Incidence of Udbatta disease under different inoculation treatments

Treatments	Infection observed in the year of inoculation (1961-62)		Infection observed in the succeeding year (1962-63) in the crop raised from the seeds collected from previous year (seeds untreated)	
	Average per cent of clump infection	Average per cent of earhead infection	Average per cent of clump infection	Average per cent of earhead infection
1. Seed inoculation	0.00	0.00	0.87	1.41
2. Preflowering inoculation	1.31	0.21	10.01	7.23
3. Post-flowering inoculation	0.00	0.00	2.09	0.48
4. Without inoculation (control)	0.94	0·04	0.55	0.12

It can be seen that preflowering inoculation has resulted in a significant increase in infection in the crop of the succeeding year indicating thereby that the earheads get infected at the time of their emergence.

B. Experiments on control measures : These consisted of varietal susceptibility trials, effect of different dates of sowing on disease incidence and effect of various fungicides, hot water treatment and solar treatment of the seeds.

Experiment No. 1 : 11 early varieties namely, T. 6517, T. 6522, CB. II, E. C. 2504, Tepa I, TKM. 6, CO. 10, PTB. 10, MTU. 9, J_1, J_2 and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

fifteen medium varieties namely, Triple cross, BK. 6, T. 141, T. 141.84, T. 141-89, T. 1879, MTU. 15, HYB. 5, EF. 86, T. 812, J_3, J_4, J_5, J_6, and J_{10} were tested under natural conditions. Out of these 26 varieties tested, 7 varieties J_1, J_2, J_3, J_4, J_5, J_6, and J_{10} obtained from Jeypore and 2 varieties namely MTU. 9 and MTU. 15 obtained from C. R. R. I, Cuttack, were grown at Pottangi during 1957-58. Three varieties Hyb. 5, EF. 86 and T. 812 obtained from Jeypore were grown at the same place during 1958-59. The rest of the 14 varieties obtained from C. R. R. I, Cuttack were grown at Jeypore during 1957-58, 1958-59 and 1959-60. After flowering, observations on incidence of the disease were taken by counting the number of affected and unaffected clumps and earheads in each variety. Varietal reaction to the disease is grouped in five categories and the varieties are placed in different groups accordingly. The data obtained are given in Table 3.

TABLE 3 : Varietal reaction to the disease

Category	Disease incidence		Varieties
	per cent of clump infection	per cent of earhead infection	
1. Highly resistant	0.00	0.00	C. B. II
2. Resistant	0·01- 1.00	0.01- 0.25	Tepal, TKM. 6, Triple cross, BK. 6, T. 141, T. 141- 84, T. 141-89, T. 1879, T. 6522 & J_{10}.
3. Moderately resistant	1.01- 2.00	0.26- 0.50	J_6, PTB. 10, T. 812.
4. Susceptible	2.01- 5.00	0.51- 1.50	T. 6517, EC. 2504, CO. 10, MTU. 9, MTU. 15, J_2 and J_4.
5. Highly susceptible	Above 5	Above 1.50	J_1, J_3, J_5, Hyb. 5, EF. 86.

Out of the varieties tried, C. B-II was found to be highly resistant and Tepa I, TKM. 6, Triple cross, BK. 6, T. 141, T. 141-84, T. 141-89, T. 1879, T. 6522 and J_{10} were found to be resistant.

Experiment No. 2 : With a view to find out the effect of different dates of sowing on disease incidence field experiments were carried out during 1958-59, 1959-60, 1960-61 and 1961-62 at Jeypore. Naturally infected seeds of J_1, obtained from Jeypore, were divided into three lots in each year of experiment and were sown in the nursery beds on three dates at an interval of 15 days. When the seedlings were one month old

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

they were transplanted in the field, replicated six times in a compact randomized block design. Observations on clump and earhead infection were recorded after flowering of the plants. The data are presented in Table 4.

TABLE 4 : Incidence of Udbatta disease in crops raised from seeds sown on different dates

Year	Dates of sowing	Average percent of clump infection	Average percent of earhead infection
1958-59	28-5-58	2.51	0.41
	12-6-58	4.67	0.99
	27-6-58	3.46	1.03
1959-60	5-6-59	1.63	0.47
	20-6-59	18.64	5.45
	4-7-59	14.29	3.77
1960-61	12-6-60	16.37	4.87
	23-6-60	16.36	4.09
	8-7-60	1.66	0.34
1961-62	25-5-61	9.31	2.68
	22-6-61	15.17	8.04
	6-7-61	3.08	1.55

It may be seen that under Jeypore conditions in period-bound variety J_1, the incidence of the disease was comparatively less if the seeds were sown very early (last week of May) or very late (1st or 2nd week of July) than the seeds sown at the usual time (2nd or 3rd week of June).

Experiment No. 3 : To determine the efficacy of seed treatment with different seed dressing fungicides, hot water and solar heat, experiments were carried out at Jeypore. Seeds of a susceptible medium duration variety J_5 were used during 1957-58 and those of another susceptible early duration variety J_1 during 1958-59 to 1960-61. Seeds were divided into eight lots. Seeds of three lots were treated separately with either Agrosan GN, Ceresan or Tillex @ 1.25 grams per 1 lb. of seeds; seeds of another three lots were soaked separately in ordinary water for 5 hours and then treated with hot water at 46° C, 50° C and 54°C for 10 minutes; seeds of the 7th lot were soaked in ordinary water for 5 hours and then exposed to sun's rays for 4 hours from 12.00 noon to 4.00 P. M. spread on a piece of cloth on cemented floor (the temperature of the cemented floor varied from 42° C to 51° C and 42·5° C to 53° C on 11-6-58 and 26-6-59 respectively, the days on which solar treatments of seeds were carried out). Seeds of the 8th lot were used as such without any treatment to serve as control. Seeds were sown in the nursery beds separately

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and when the seedlings were one month old, they were transplanted in the field replicated three times in a compact randomized block. Observations on incidence of the disease were taken after flowering of the plants and the data are given in Table 5.

TABLE 5 : Incidence of the disease under different seed treatments

Treatments	Average percent of clump infection				Average percent of earhead infection			
	57-58	58-59	59-60	60-61	57-58	58-59	59-60	60-61
1. Agrosan GN	7.98	18.31	12.63	4.63	4.90	10.30	5.76	2.84
2. Ceresan	7.18	21.74	8.37	4.49	4.09	11.91	3.96	2.46
3. Tillex	8·08	17.64	10.86	6.38	4.44	10.94	5.71	3.59
4. Hot water-46°C	—	—	7.69	4.26	—	—	3.67	2.42
5. -do- 50°C	1.55	3.42	0.76	0.00	0.69	1.59	0.19	0.00
6. -do- 54°C	1.15	1.88	0.00	0.00	0.74	0.93	0.00	0.00
7. Solar treatment	—	12.93	2.84	—	—	6.91	0.66	—
8. Control	8.31	15.33	10.09	6.37	4.22	8.56	4.89	3.29
C. D· (0.05)	4.34	12.32	11.71	1.21	2.13	4.76	1.57	2.12
(0.01)	6.17	—	—	3.18	3.04	6.57	2.18	—

In the treatment with hot water at 50° C and 54° C, the decrease in percentage of earhead infection was highly significant in all the four years of trial; the percentage of clump infection, however, did not show significant difference over the control for two seasons 1958-59 and 1959-60 in spite of their lowest value for the years. Solar treatment also gave highly significant reduction in the disease incidence during 1959-60. Agrosan GN, Ceresan and hot water treatment at 46° C showed significant decrease in the incidence of the disease at 5 per cent level for one year only. Hot water treatments at 50° C and 54° C appear to be highly effective in controlling the disease.

DISCUSSION : The mode of infection of the Udbatta disease appears to be through seeds. The effectiveness of hot water treatments over that of fungicidal treatments show that the pathogen is perhaps internally seed-borne. Fresh infection may be taking place at the time of flower opening and may be carried through the seed to the seedling. From germination to flowering, the infection might be present inside the tissues in a systemic manner as suggested by Tai and Siang (1948) and Govindu

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and Thirumalachar (1954). As a matter of fact, in susceptible varieties, the symptoms of infection could be detected even before flower emergence as has been indicated earlier. The prevalence of the disease in higher elevations than in plains points out that eco-climatic conditions prevaling in those areas are more favourable for the completion of the life cycle of the fungus than in the plains.

When infected seeds from Jeypore were brought just before sowing time and sown at Bhubaneswar, where the disease is not prevalent, some plants yielded infected earheads which suggest that the condition for survival of the pathogen in a systemic manner inside the host are not so critical as the conditions determining pre-infection and flowering and survival of the pathogen in the seed. Besides, *Ephelis* sp. has been recorded on a large number of grasses, as reported by Sydow and Butler (1916), Venkatkrishnaya (1946, 1952), Govindu and Thirumalachar (1954), Rao and Reddy (1956) and Mohanty and Mohanty (1957). Some of these possibly, may be serving as a source of inoculum for fresh infection of rice plants.

SUMMARY

Symptoms of Udbatta disease of rice caused by *Ephelis oryzae* Sydow have been described. The disease was found to be internally seed-borne. The disease was not found to be soil-borne.

The infection appears to be initiated at the time of emergence of earheads.

Out of the 26 varieties tested for varietal suceptibility and resistance under natural conditions, C. B-II was found to be highly resistant and Tepa-I, Triple cross, T. 141, T. 141-84, T. 141-89, T. 1879, T. 6522 and J_{10} were found to be resistant.

The incidence of the disease was found to be comparatively less in very early or very late sown crops than in case of those sown in normal time for period-bound varieties.

Hot water treatment of the seeds at 50° C and 54° C for 10 minutes before sowing was found to be effective in controlling the disease. Solar treatment of the seeds was also found to be promising.

ACKNOWLEDGEMENT : The author is deeply indebted to Dr. U. N. Mohanty, Principal, Utkal Krushi Mahabidyalaya, Bhubaneswar for his interest, encouragement and valuable suggestions during the course of this investigation. He is also thankful to Dr. B. Parija, Professor of Botany and the staff of Rice Research Station, Jeypore and Agricultural Farm, Pottangi for their help in conducting field experiments. The author is grateful to the Director of Central Rice Research Station, Cuttack for the supply of seed material and to Sri S. Y. Padmanabhan, Mycologist, C. R. R. I. Cuttack for going through the manuscript and helpful criticism. Thanks are also due to Sri S. K. Addy, Sri S. N. Das, Sri B. C. Behera, Sri G. C. Rath and Sri S. N. Pattanaik of Mycology Section for their help in preparation of the manuscript.

Mycology Section,
Utkal Krushi Mahabidyalaya,
Bhubaneswar.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

GOVINDU, H. C. AND M. J. THIRUMALACHAR (1954) *Ephelis* on *Sorghum halepense* in Mysore. *Science*, **119** : 288—289.

KAMAT, M. N. AND M. K. PATEL (1951) Notes on two important plant diseases of Bombay State. *Plant Prot. Bull.*, **3** : 16.

MOHANTY, U. N. AND N. N. MOHANTY (1957) *Ephelis* on some new hosts. *Sci.* and *Cult.*, **22** : 8.

RAO, P. G. AND G. S. REDDY (1956) Two new records of *Ephelis* on grass hosts. *Sci. and Cult.*, **21** : 669-671.

*SYDOW H., P. SYDOW AND E. J. BUTLER (1916) *Fungi Indiae orientalis V. Ann. Mycol. Berl.*, **14** : 177—220.

*TAI, F. L. AND W. N. SIANG (1948) I-Chu-hsiang of rice caused by *Ephelis oryzae* Sydow in Yunnan. *Acta. Agric.*, **1** : 125—131.

VENKATAKRISHNAYA, N. S. (1946) *Ephelis* on two new hosts. *curr. Sci.*, **15** : 260—261.

VENKATAKRISHNAYA, N. S. (1952) Ephelis on *Echinochloa crus-galli* Beauv. and *Setaria italica* Beauv. *Phytopathology*, **42** : 634—636.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SOME INTERESTING FLESHY FUNGI FROM HIMACHAL PRADESH

H. S. Sohi, S. Kumar and P. K. Seth

(Accepted for publication November 17, 1964)

A survey for collection of different edible mushrooms from various localities of Himachal Pradesh is in progress for sometime past. A large number of fleshy fungi have been collected and the specimens have been deposited in the Herbarium of the Plant Pathology Section, Department of Agriculture, Himachal Pradesh and their respective numbers are indicated in the text.

1. *Scleroderma lycoperdoides* Schw.

Fructifications gregarious or caespitose, body 1.3-4.0 cm. broad, surface light brown, dotted all over with minute dark brown separated scales, globose to sub-globose, turbinate or depressed globose, sessile, flat below and abruptly rooted by a stout embedded stalk of varying length which is soon dissipated into few strong stout strands or into many small fibers (Fig. 1). In some cases pseudo-stalks present. *Peridium* single, thick when fresh and immature, becoming thinner with maturity, firm, obscurely defined, the inner layer becoming fibrous like the trama and continuous with it. *Capillitium* absent, in mature specimens only the outer thin layer of a leathery texture remains intact, glebal chambers. very small, delicate and straight hyphae constitute the thin plates of the glebal chambers. *Spores* blackish purple in a mass, deep purplish brown when observed singly, spherical, not reticulated, conspicuously spiny (Fig. 12), asperulate, 6·9-9·3 μ in diameter. *Dehiscence* irregular through pores made by the grubs or other insects mostly on the upper portion of the fructifications, during later stages the upper portion of the fructification ruptures irregularly while the lower portions remain intact in the form of an empty saucer.

Collected on soil in the forest areas of Solan (5,000' m. s. l.) during July, 1963, S. Kumar, H. C. No. 5011. In another collection (No. 5011 B.) made from Solan during July, 1963, the spores were reticulate (Fig. 10). Hence it was identified as *S. lycoperdoides* var. *reticulatum*.

2. *Geaster floriformis* Vitt.

Fructifications small, 1.8-2.6 cm. in height, 1·7-2.5 cm. in diameter, submerged until mature. *Peridium* consisting of two distinct parts, outer peridium thick, splitting at maturity into 8-10 star-like unequal rays (Fig. 2 a), outer layer thick, hard when dry, whitish, smooth, inner layer fleshy, whitish to brownish, cracked when dry; inner peridium persistent forming a definite spore sac with a single apical mouth, pale in early stages, covered at first with fine granules or furfuraceous particles which soon wear away in large parts; rays hygroscopic, covered at first with earth held on by the mycelium. *Peristome* only a puncture or slit with short radiating fissures with no well defined area around it. *Spores*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

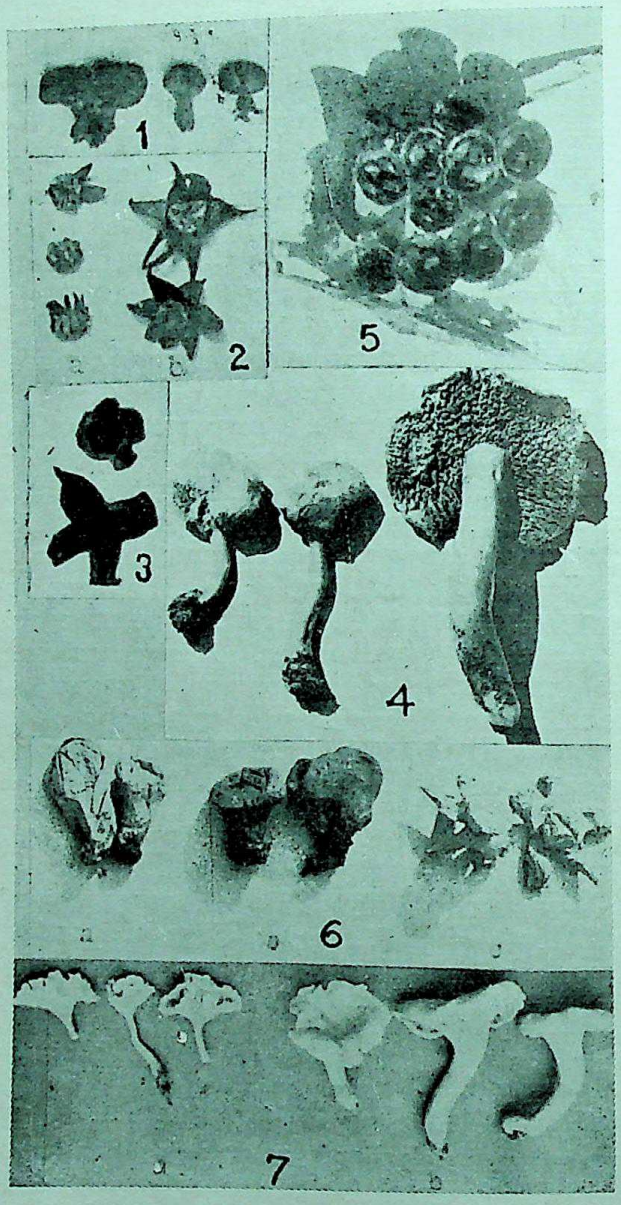
Digitized by Arya Samaj Foundation Chennai and eGangotri

distinctly warted, spherical (Fig. 11), 5.8-6.9 μ in diameter. *Capillitium* consisting of slightly curved threads, thick walled, upto 6.9 μ thick.

Collected on sandy soil in the forest areas of Gopalpur (6,900' m. s. l.) during March, 1963, S. Kumar, H. C. No. 5012.

3. *Geaster limbatus* Fr.

Collected on soil covered with dead decaying organic matter from Khadrala (9, 400' m.s.l.) and Nichar (7,000' m.s.l.) during October, 1962, P. K. Seth, H. C. No. 5013.



Figs. 1—7. 1. *Scleroderma lycoperdoides*. 2a. *Geaster floriformis*. 2b. *Geaster morgani*. 3. *Geaster triplex* Jung. 4. *Hydnum* repandum var. *album*. 5. *Cyathus stercoreus*. 6a. *Lycoperdon gemmatum*. 6b. *Lycoperdon atropurpureum*. 6c. *Geaster limbatus*. 7a. *Cantha rellus minor*. 7b. *Cantharellus cibarius*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

4. *Geaster morganii* Lloyd

Fructifications large, broadly bulb shaped, pointed with a short or long point (2-5 mm. in length); Outer peridium splitting into 6-8 rays with long acuminate tips which become revolute under the convex arched base (Fig. 2 b), the outer layer tending to crack and peel off in flakes, inner fleshy layer dark brown to blackish, cracking; inner peridium thin, sub-spherical forming a spore sac, usually compressed when dry, minutely granular, greyish to brown in colour. *Peristome* forming a narrowly conical papilla with the sides crumpled all over or toward the top forming a pseudosulcate peristome with lacerated tip, not conspicuously outlined. *Columella* clavate and persistent. *Spores* smoky brown in a mass, violaceous brown under the microscope, spherical, distinctly warted (Fig. 9), 3.4-40 μ. *Capillitium* consisting of unbranched threads with wavy margins.

Collected on the ground from the forest areas of Mashobra (7,500' m.s.l.) during September, 1963, S. Kumar, H.C. No. 5014.

5. *Geaster triplex* Jung.

Collected on soil in the forest areas of Nichar (7,000' m.s.l.) during April, 1963, S. Kumar, H. C. No. 5015.

6. *Lycoperdon atropurpureum* Vitt.

Fructifications scattered, 2.4-5.0 cm. high and 2.2-3.6 cm. broad, usually depressed globose or nearly pyriform, sometimes obovate to turbinate, with large distinct stalk-like base (Fig. 6 b), greyish tan to buff or leather coloured, cortex covered with very delicate hair like spicules and granules. These are persistent in fresh collections but absent in old ones leaving the cortex smooth. *Gleba* at maturity deep smoky purple. Sub-gleba occupying the stem like base which is usually more than half of the whole fructification, chambered. *Capillitium* composed of long brown, unpitted, branched threads, attenuated at the ends, 4.6-6.9 μ in diameter at thick places. *Spores* deep smoky purple, spherical, 3.5-5.8 μ in diameter, spiny, pedicellate (Fig. 8); pedicel not persistent.

Collected on soil covered with dead decaying organic matter from Gopalpur (6,900' m. s. l.) during August, 1963, S. Kumar, H. C. No. 5016.

7. *Lycoperdon gemmatum* Batsch

Collected on soil covered with dead decaying organic matter at Gopalpur (6,900' m. s. l.), during September, 1963,S. Kumar, H. C. No. 5017.

8. *Cyathus stercerous* (Schw.) De Toni

Collected on rotten wheat straw at the Government Agricultural Farm, Solan (5,000' m. s. l.) during July, 1962, P. K. Seth and S. Kumar, H. C. No. 5018.

It has already been reported from Poona (Bombay) on manure heaps; Gurdaspur (Punjab-I) on dung cakes; Chamba (H. P.) on manure but the present specimen was collected on rotten wheat straw.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

9. *Tylostoma verrucosum* Morg.

Collected on the ground covered with dead decaying organic matter from the forest areas of Gopalpur (6,900' m. s. l.) during October 1963, S. Kumar, H. C. No. 5019. Reported from Gurdaspur (Punjab I) on ground.

10. *Melanogaster ambiguus* Tul.

Collected on soil from the forest areas of Solan (5,000' m. s. l.) during August, 1963, S. Kumar, H. C. No. 5020. Reported from Dalhousie (Punjab-I) on ground.

11. *Cantherlleus cibarius* Fr.

Collected on soil covered with dead decaying organic matter during August, 1962 from Solan (5,000' m. s. l.), P. K. Seth, H. C. No. 5021. Reported on ground from Arnigodh, Mussoorie (U. P.) under *Pinus longifolia*.

12. *Cantharellus minor* Peck

Fructifications gregarious, stipitate, fleshy, pileate, pileus typically bell-shaped when young, convex or nearly plane, often depressed or with a pit at the centre when mature (Fig. 7 a), thin, smooth above, yellow with a reddish tinge, 1.25-2.5 cm. in diameter, margin wavy or irregular. *Stem* slender, smooth, solid when young, hollow when old, reddish to yellowish in colour, erect or bending, uniform in diameter below, slightly expanded above gradually merging into the pileus, 1.5-3.0 cm. in length. *Hymenium* composed of numerous gills, exposed, gills far apart, decurrent, forked, yellow with a reddish tinge, very conspicuous in the lower part. *Basidia* clavate, hyaline, non-septate, bearing 4 terminal long sterigmata (Fig. 13 a). *Basidiospores* hyaline, obovate (Fig. 13 b), 5.8-8.1 $\times$ 9.6 μ.

Collected on soil covered with dead decaying organic matter at Solan (5,000' m.s.l.) during August, 1962, P. K. Seth & S. Kumar H. C. No. 5022.

13. *Amanita vaginata* (Bull. ex. Fr.) Vitt.

Collected on soil in the forest areas of Solan (5,000' m.s.l.) during June, 1963, S. Kumar, H. C. No. 5023. Reported from Khasi Hills (Assam).

14. *Macrolepiota procera* (Scop. ex. Fr.) Sing.

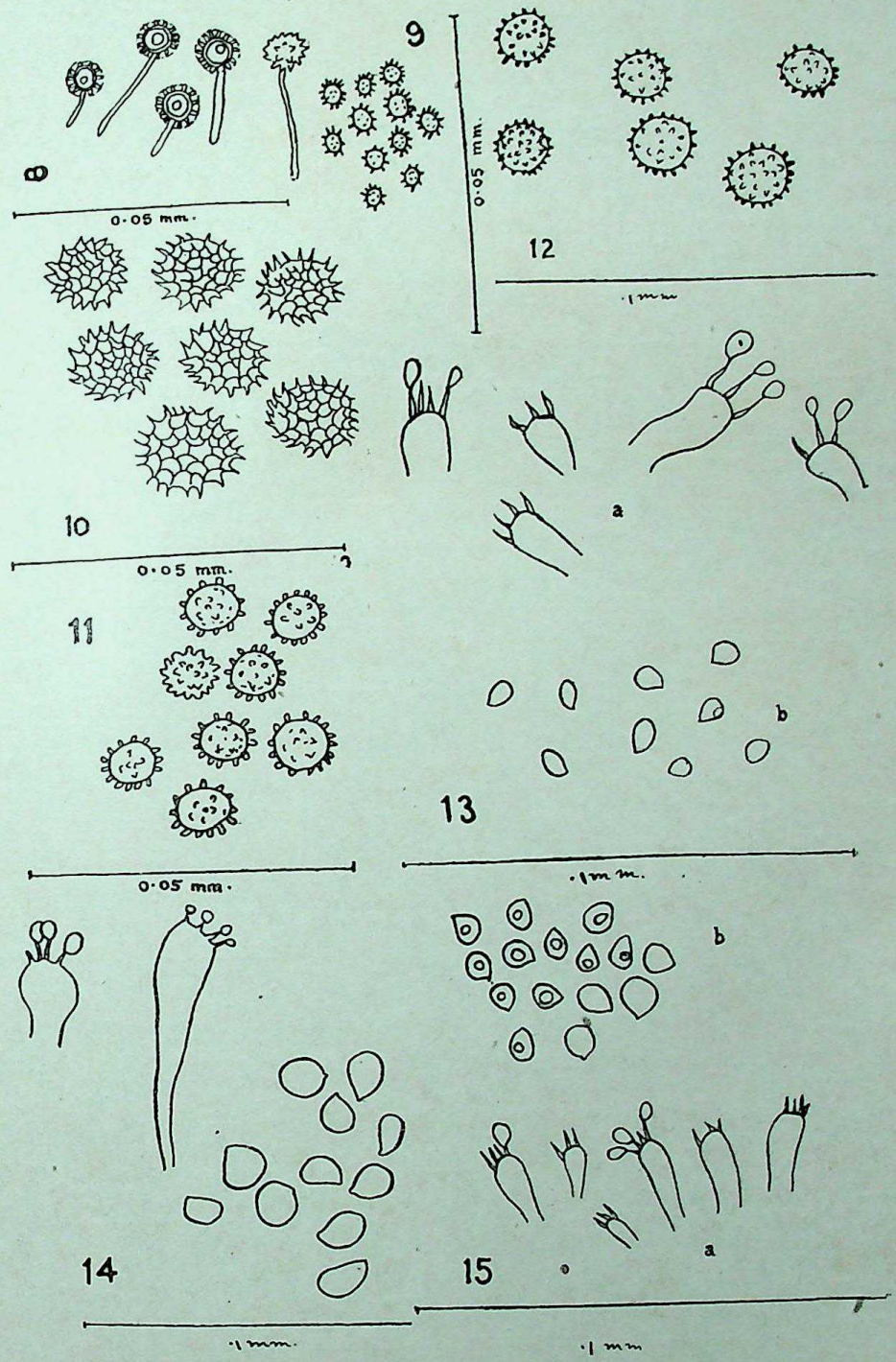
Collected on dead decaying organic matter in the forest areas and pastures of Solan (5,000' m.s.l.) during July, 1963, S. Kumar, H. C. No. 5024. Reported from Saharanpur (U. P.) on the ground under *Ficus carica*.

15. *Hydnum repandum* L. var. *album* (Quel.) Rea

Fructifications large, scattered, stipitate, cap fleshy, tender, upto 5.5 cm. in diameter, irregular, convex or depressed at the centre (Fig. 4), white to creamish, upto 5 mm. thick. *Stem* upto 6.0 cm. long, central, variable in thickness, enlarged below or above or at both places, white to creamish and solid. *Spines* white to creamish, upto 6 mm. long, pointed. *Basidia* clavate, non-septate, bearing 4 long apical sterigmata (Fig, 15 a). *Basidiospores* hyaline, smooth, sub-globose (Fig. 15 b), 7.1-8.3 $\times$ 6.9-7.1 μ and with a single large oil drop.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 8—15. 8. *Lycoperdon atropurpureum*, Spores. 9. *Geaster morganii*, Spores. 10. *Scleroderma lycoperdoides* var. *reticulatum*, Spores. 11. *Geaster floriformis* Spores. 12. *Scleroderma lycoperdoides* Spores. 13. *Cantharellus minor* : (a) Basidia with Basidiospores. (b) Basidiospores. 14. *Amanita vaginata* : (a) Basidia with Basidiospores. (b) Basidiospores. 15. *Hydnum repandum* var. *album* : (a) Basidia with Basidiospores. (b) Basidiospores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Collected on soil covered with dead decaying organic matter from Solan (5,000' m.s.l.) during August, 1962, P. K. Seth, H. C. No. 5025. *Hydnum repandum* is already reported from Arnigodh, Mussoorie (Uttar Pradesh) on soil, but *H. repandum* var. *album* is a new record for India.

SUMMARY

15 fleshy fungi have been described, out of which *Scleroderma lycoperdoides* Schw., *S. lycoperdoides* Schw. var. *reticulatum*, *Geaster floriformis* Vitt., *G. morganii* Lloyd, *Lycoperdon atropurpureum* Vitt., *Cantharellus minor* Peck and *Hydnum repandum* L. var. *album* (Quel.) Rea are new records for India while *Geaster limbatus* Fr., *G. triplex* Jung, *Lycoperdon gemmatum* Batsch, *Tylostoma verrucosum* Morg., *Melanogaster ambiguus* Tul., *Cantharallus cibarius* Fr., *Amanita vaginata* (Bull. ex. Fr.) Vitt. and *Macrolepiota procera* (Scop. ex. Fr.) Sing. are being recorded for the first time in Himachal Pradesh. *Macrolepiota procera*, *Cantharellus minor* and *C. cibarius* are edible.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh for his keen interest in the work and for the facilities provided during the course of these studies. Grateful thanks are also due to Indian Council Agricultural Research for partly financing the Mushroom Cultivation Scheme.

Plant Pathology Section,
Himachal Pradesh, Solan.

REFERENCES

AHMAD, S. (1939) Higher fungi of the Punjab plains. I. The *Gasteromycetes*. *J. Indian bot. Soc.* **18** : 47—58.

——— (1940) Higher Fungi of the Punjab plains. II. The *Gasteromycetes*. *J. Indian bot. Soc.*, **18** : 169—177.

——— (1941) Higher Fungi of the Punjab plains III. The *Gasteromycetes*. *J. Indian bot. Soc.*, **20** : 135—143.

——— (1942) *Gasteromycetes* of the North Western Himalayas-II. *J. Indian bot. Soc.*, **21** : 283—293.

COKER, W. C. AND A. H. BEERS, (1951) *The Stipitate Hydnums of the Eastern United States*. The University of North Carolina Press, Chapel Hill, U. S. A.

——— AND J. N. COUCH (1928) *The Gasteromycetes of the Eastern United States and Canada*. Univ. North Carolina Press.

SINGER, R. (1962) *The Agaricales in Modern Taxanomy*. J. Cramer, Weinheim, Germany.

VASUDEVA, R. S. (1960) *The Fungi of India*. Indian Council of Agricultural Research New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

OCCURRENCE OF TOBACCO MILD MOTTLE VIRUS ON TOBACCO

H. N. VERMA

(Accepted for publication November 20, 1964)

A disease characterized by dark green mosaic and with occasional necrotic spots on leaves was observed in some tobacco (*N. tabacum* var. Natu Cigarette vala) plants growing in the farm. When the sap from diseased leaves was inoculated on this variety and also on *N. glutinosa* L., it produced dark green mosaic symptoms with occasional necrotic spots on the former and severe systemic mosaic symptoms with deformation of leaves on the latter (Figs. 1 and 2). Detailed studies of the virus were undertaken to determine its host range, physical and serological properties.

MATERIAL AND METHODS: The virus strain was cultured on *N. glutinosa* and used when needed. Infected leaves were macerated in a pestle and mortar, adding 1 ml. of distilled water to 1 gram of leaves. The pulp obtained was squeezed through muslin cloth and the sap was centrifuged at 3000 r. p. m. for 15 minutes to obtain a clear supernatant which was used as inoculum. Inoculations were done by gently rubbing the upper surface of leaves with forefinger wet with inoculum. Prior to inoculation leaves were dusted with carborundum powder. Test plants were kept in insect proof conditions in a glass house.

Antiserum was prepared by injecting rabbit with virus which was partially purified by centrifuging the infective sap at 8,000 r. p. m. for 15 minutes. Five intravenous injections were given at intervals of 3-4 days. The rabbit was bled after 15 days of the last injection and the antiserum was stored in the freezing chamber of the refrigerator with 0.5 per cent phenol. The sap (antigen) for serological tests was clarified by heating the sap to 40°C for 10 minutes and centrifuging it at 8,000 r.p.m. for 15 minutes. The dilutions of the antiserum and antigen were made in 0.85 per cent NaCl.

Precipitin tests were made by mixing 1 ml. of antigen and 1 millilitre of antiserum in tubes 8 mm. in diameter which were later incubated in a water bath at 40°C.

RESULTS: The virus was confined only to family Solanaceae and all attempts to infect plants of other families were unsuccessful. The virus was easily sap transmissible and usually gave 100 per cent infection with sap inoculation. The insect vectors, if any could not be found. *Aphis rhamni* Boyer., *Aphis gossypii* Glover, *Myzus persicae* Sulz. and *Aphis rumicus* did not transmit the virus.

Host range of the virus: N. tabacum var. *Natu Cigarette vala*— Mild mosaic symptoms were observed on young growing leaves 10-12 days after inoculation. Sometimes yellowish spots also developed on the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

middle-aged systemically infected leaves. In later stages of infection dark green blisters appeared on the newly emerging leaves. The general appearance of symptoms was of mild mosaic and mottling. The size and growth of plant was only slightly affected.

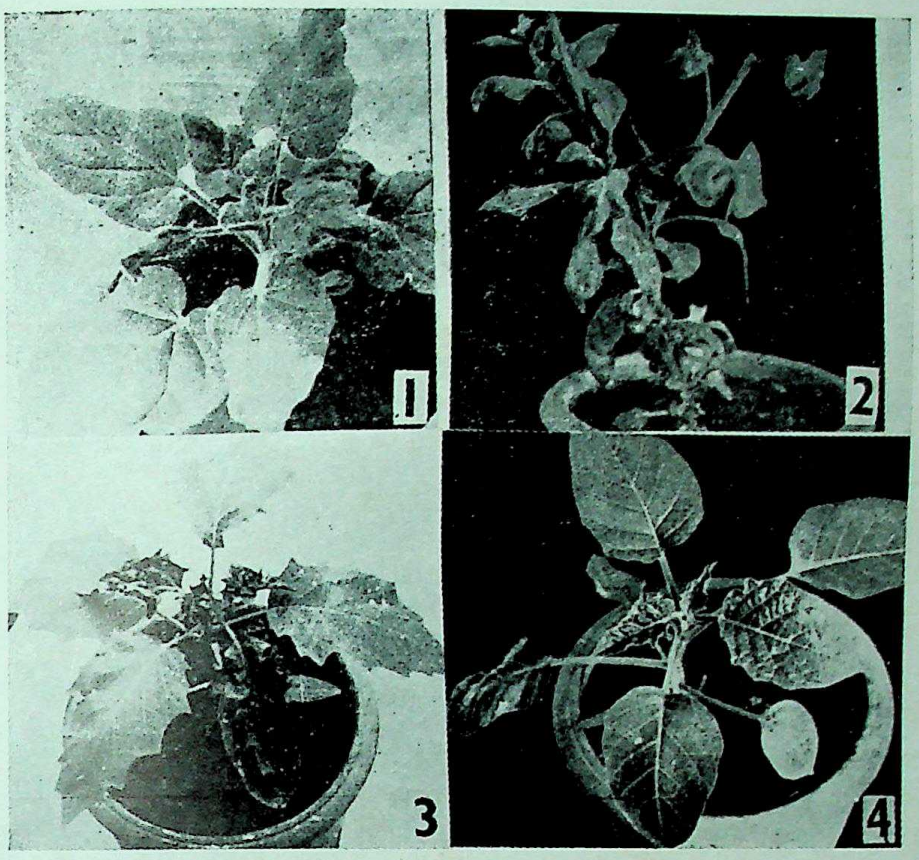
Symptoms developed on plants other than *N. tabacum* var. Natu Cigarette vala are summarized in Table 1.

TABLE 1 : Summary of the symptoms developing on different host plants

Family	Host plant	Characteristic symptoms.
Solanaceae	*N. tabacum* var. N. P. 31	Mosaic and also yellow necrotic spot on newly emerging leaves.
,,	*N. tabacum* var. Harrison's special	Vein clearing followed by mosaic. Newly emerging leaves were slightly deformed.
,,	*N. tabacum* var. Chatam	Mild green mosaic appeared 8-10 days after inoculation.
,,	*N. glutinosa* L.	Vein clearing followed by bright green mosaic. Later on leaves were deformed variously and became linear and strap shaped (Figs. 1 and 2).
,,	*N. trigonophylla* L.	Same as in *N. glutinosa*, excepting the deformity of leaves was not so much pronounced.
,,	*N. rustica* var. C-302	Prominent mottling appeared after 2 weeks of inoculation and this later on disappeared and plants recovered.
,,	*N. rustica* var. NPS 219	Mottling sometimes followed by necrosis. Symptoms disappeared after a month and plant recovered.
,,	*Physalis peruviana* L.	Mosaic becoming mild in later stages.
,,	*D. stramonium* L.	After a week or so of virus inoculation the newly emerging systemically infected leaves showed upward rolling and puckering of leaves. Dark green raised and sunken areas also appeared. Flowering and fruiting was checked (Fig. 3).
,,	*D. metel* L.	Vein clearing and mild mottling, later on symptoms disappeared and plants recovered (Fig. 4).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 1—4. 1 and 2 Young and old plant of *N. glutinosa* showing vein clearing, mosaic mottling and variously deformed leaves. 3. *D. stramonium* showing mosaic mottling and puckering of leaves. 4. *D. metel* showing vein clearing and mild mottling of leaves.

The following plants could not be infected :

Solanum nigrum L.; *Solanum melongena* L.; *Petunia hybrida* Vilm.; *Lycopersicon esculentum* Mill.; *Spinacia oleracea* L.; *Brassica oleracea* L.; *Beta vulgaris* L.; *Chenopodium album* L.; *Chenopodium ambrosioides* L.; *Luffa aegyptica* Mill.; *Cucumis melo* L.; *Cucurbita maxima* L.; *Vigna sinensis* Savi ; *Phaseolus vulgaris* L. var. Kentucky wonder and Giant stringless ; *Tropaeolum majus* L.; *Malva sylvestris* L.; *Anchusa officinalis* L.; *Dahlia pinnata* Cav.; *Cineraria* sp.; *Penstemon* sp.

Properties in vitro : Thermal inactivation point—The virus withstood 10 minutes exposure to 50°C and was inactivated at 60°C.

Longevity in vitro : The virus retained infectivity in expressed sap at room temperature (28-30°C) for 56 hours only and at 0°C for 10 days.

Dilution end point : Virus infectivity was retained at a dilution of 1/100 but not at 1/1000.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Serological properties : Antiserum did not react with TMV antigen. However positive reactions were obtained with a strain of tobacco etch virus (Varma and Lal, 1964) and datura virus 3 (Verma and Verma, 1963) showing thereby some serological relationship with the virus. These viruses also produce systemic symptoms on *N. glutinosa*, although they differ widely in host range and host reactions.

TABLE 2 : Precipitin tests with homologous antigens at different dilutions of antiserum and antigen (after 1 hour)

	Dilution of Antigen					
Dilution of antiserum	1/1	1/10	1/50	1/100	1/150	1/200
1/10	+++++	++++++	+++	++	++
1/40	+++	++	+	+	—	—
1/80	++++	++	+	+	—	—
1/160	+++	++	—	—	—	—
1/250	++	—	—	—	—	—
1/320	+	—	—	—	—	—
Control : Healthy *N. glutinosa*						
1/10	++	++	+	—	—	—
1/40	+	—	—	—	—	—
1/80	—	—	—	—	—	—

— = no reaction ; + = faint precipitate ; ++ = slight but definite precipitate.

+++= moderate precipitate ; ++++ or +++++= heavy precipitate.

Antigen controls and normal serum with 0.85 per cent NaCl gave negative results.

When the antiserum was titrated against antigen (clarified sap of *N. glutinosa*) it gave precipitation end point of 1/250 (dilution of antiserum) with standard antigen and 1/40 with healthy sap of *N. glutinosa*. The precipitate formed was of floccular type. The optimum dilution of antiserum for maximum precipitation was 1/80 and the time required for complete precipitation was 1 hour. With diluted antigens the precipitation end point was decreased (Table 2).

DISCUSSION : The data indicated that in its properties *e.g.*, dilution end point, thermal inactivation point and some of its host reactions it closely resembled the tobacco mottle virus, one of the viruses causing the tobacco rosette complex (Smith, 1946) but it differed in its inability to produce visible symptoms on tomato and in producing symptoms on *D. stramonium* which did not disappear in the advanced stages of infection. The longevity *in vitro* of the present virus was 56 hours as compared to two days of Tobacco mottle virus. The virus had been reported from Southern Rhodesia and Nyasaland and there is no report of this virus from anywhere else so far.

The serological studies indicated its relationship with a strain of *Datura virus 3* (Verma and Verma, 1963) and a strain of tobacco etch virus

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Verma and Lal, 1964), but as regards the symptomatology on different hosts and physical properties the present virus differed widely from these strains.

Considering its close relationship with the tobacco mottle virus the present virus has been named as *Tobacco mild mottle virus* and may be treated as a distinctive strain of Tobacco mottle virus.

SUMMARY

The wild tobacco mild mottle virus (TMMV) was found occurring on *N. tabacum* var. Natu Cigarette vala in Lucknow. It was transmitted by sap inoculation to different varieties of *N. tabacum*, *N. glutinosa*, *N. trigonophylla*, *N. rustica* var. C-302 and NPS 219, two species of *Datura* (*D. metel* L. and *D. stramonium* L.) and *Physalis peruviana*. The virus was inactivated in 10 minutes at 60°C, at a dilution of 1/1000 and in 56 hours at room temperature (28-30°C). A visible floccular precipitate was obtained with the standard antigen upto a dilution of 1/250 of antiserum and with 1/10 antiserum upto a dilution of 1/200 of antigen. Serologically, it was found related to a strain of tobacco etch virus and Datura virus 3 but not to tobacco mosaic virus. The virus was quite related to tobacco mottle virus in host range, host reaction and physical properties.

ACKNOWLEDGEMENT : The author is deeply grateful to Dr. G. S. Verma for his valuable guidance and keen interest during the course of the investigation. Thanks are also due to the Council of Scientific and Industrial Research for the award of Junior Research Fellowship during the tenure of which this work was completed.

Botany Department,
Lucknow University,
Lucknow.

REFERENCES

SMITH, K. M. (1946) Tobacco rosette; a complex virus disease. *Parasitology*, **37** : 21-24.

SMITH, K. M. (1948) Transmission of plant virus complex by aphids. *Parasitology*, **37** : 131-134.

SMITH, K. M. (1957) *A text book of plant virus diseases*. J. and A. Churchill Ltd.

VERMA, G. S. AND H. N. VERMA, (1963) Enation mosaic virus disease of *Datura metel* L. *Indian Phytopath.*, **16** : 368-373.

VERMA, G. S. AND RASHMI LAL (1964) Occurrence of a new strain of a tobacco *et al.* virus on brinjal (*solanum melongena* L.) *Proc. Indian Sci. Congr.* **51 & 52 III** : 328.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EVALUATION OF FUNGICIDES FOR THE CONTROL OF BLACK ROT OF SWEET ORANGE

SUSHIL KUMAR AND RAJENDRA K. GROVER

(Accepted for publication November 24, 1964)

Alternaria citri Pierce, causal organism of black rot of sweet orange (*Citrus sinensis* Osbeck), was first reported from India by Uppal *et al.*, (1935). During November-December, 1963, serious epiphytotics broke out in northern and north-western Punjab, especially in Abohar or Ferozepur district. Premature fall of fruits led to over 50 per cent losses in some areas.

First symptoms of the disease are seen when the fruit approaches maturity. A black circular spot of the size of navel-end becomes visible. Under favourable conditions, the outer skin surrounding the navel-end develops a pinkish zone which gradually progresses both in colour and area. A pronounced rotting of the central core ensues which extends towards the stalk-end and results in a pre-mature fruit drop. Late infected oranges rot during storage and transit.

The severity of this disease on sweet oranges has never been reported before from India, and hence no control measures are known for this disease. Investigations were, therefore, undertaken to evaluate some common fungicides against the pathogen in the laboratory, in order to devise suitable field-spray schedules to control the recurrence of the disease. The results are reported in the present paper.

MATERIAL AND METHODS: The pathogen was isolated from diseased sweet orange fruits, and its pathogenicity established by inoculating mature oranges. Single spore isolates were used in fungicidal assays.

The fungicides used were: Cycloheximide (Actidione, Beta-[2-(3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide ; technical sample) ; Copper sulphate ; Dithane Z-78 (Zinc ethylene bis-dithiocarbamate ; 78 per cent proprietary fungicide); Dodine (Cyprex, n-dodecyl-guanidine acetate ; technical sample) ; Micop-50 (50 per cent copper W. P.); Phaltan (n-trichloromethyl-thiophthalimide ; technical sample); Phygon (Diclone, 2, 3-dichloro-1, 4-naphthoquinone ; technical sample) ; and Wettable sulphur.

For evaluating the fungicides *in vitro*, the test-tube dilution technique for use with slide germination tests (American Phytopathological Society, Committee on Standardization of Fungicidal tests, 1947), was employed. Spores of the fungus were obtained from 8-10-day-old cultures growing on Czapek's agar at 28°C. A spore suspension, 1×10^3/ml concentration, was used in all tests. Observations were taken after 24 hours at 26-28°C. A total of 500 spores from five replicates was counted for germination in each treatment and, on the basis of Dosage-response curves drawn on log-concentration probit graph (Dimond *et al.*, 1941 ; Horsfall, 1956), the ED_{50} of each chemical was determined.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RESULTS : *In vitro toxicity of the fungicides* : It will be seen from Fig. 1 that copper sulphate, dodine, phaltan and cycloheximide possessed low ED_{50} values (1.25, 2.0, 2.5, and 4.5 μg/ml., respectively), indicating their greater toxicity against the pathogen *in vitro* as compared to other fungicides. The least effective fungicides were Wettable sulphur, Micop W-50, and Dithane Z-78.

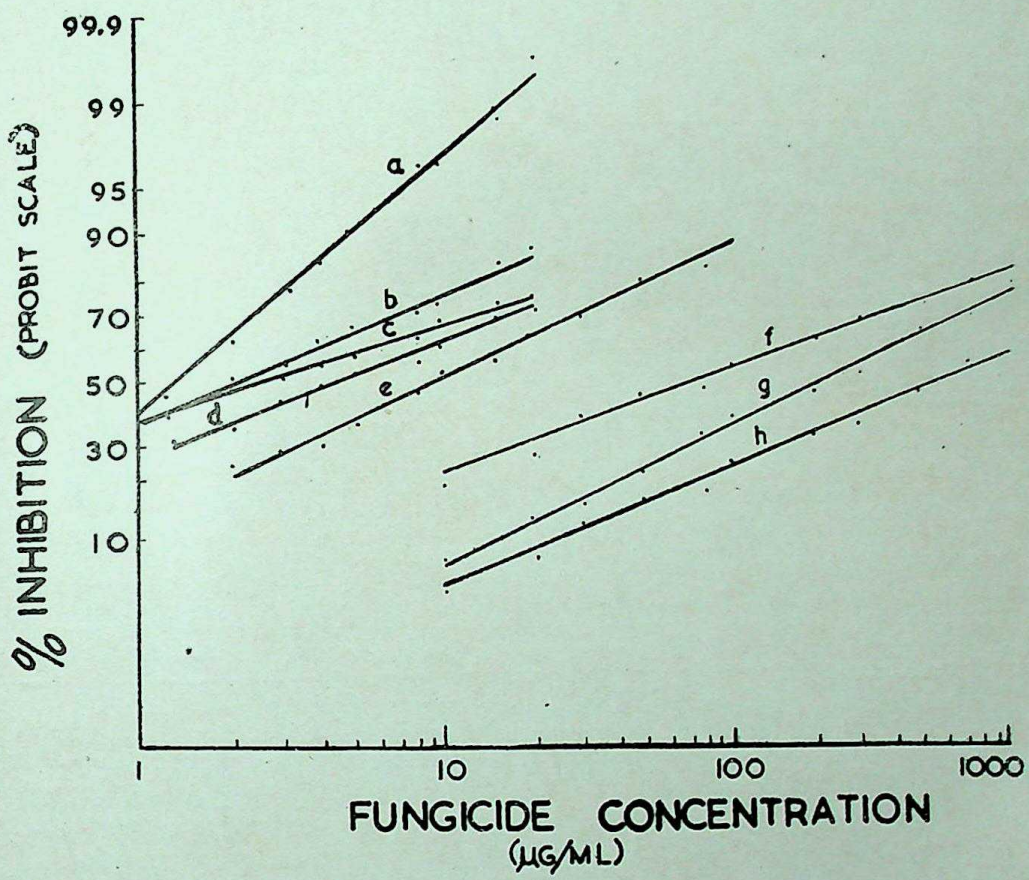


Fig. 1. Dosage response curves of *Alternaria citri* spore germination against—(a) Cu SO_4; (b) Dodine; (c) Phaltan; (d) Cycloheximide; (e) Phygon; (f) Dithane Z-78; (g) Micop W-50; and (h) Wettable sulphur.

Efficacy of the fungicides against the disease : In view of the foregoing, the said four chemicals were used in further studies on the control of black rot of sweet oranges. Bordeaux mixture was included as a standard test fungicide. The efficacy of the fungicides against the disease was studied on sweet oranges var. Jaffa, artificially inoculated in the laboratory (after Eckert and Kolbezen, 1963). Two cross scratches about 3-4 cm. each, were made at the opposite sides with a sterile saw on the surface sterilized oranges under aseptic conditions. These oranges were then sprayed with two concentrations of each fungicide with a de Vilbiss atomizer till run-off of the fungicides. A spore suspension prepared in the manner described above, was used for inoculation, 24 hours before or after treatment with the fungicides. Four replicates of five fruits in each, were used per treatment. The inoculated fruits were kept in a moist chamber at 28-30°C for 10 days. At the end of this period the extent of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the rot as mean diameter of the affected tissue was measured. The results are presented in Table 1.

TABLE 1 : Effect of five fungicides on black rot of sweet oranges

Fungicide	Concentration	Mean rot (mm) after 10 days of inoculation		Remarks
		Pre-inoculation	Post-inoculation	
Bordeaux mixture	5:5:100	10±3	20±3	Skin not
	4:4:50	3±1	10±2	much affected
Copper sulphate	1.25 ppm	11±2	8±2	Skin tough
	2.50 ,,	5±1	2±1	and leathery
Cycloheximide	4.50 ,,	24±3	30±4	Skin softened
	9.00 ,,	11±2	18±3	
Dodine	2.00 ,,	26±3	21±3	Skin tough
	4.00 ,,	12±2	10±2	
Phaltan	2.50 ,,	7±2	6±2	Skin normal
	5.00 ,,	2±1	1±1	
Control		38±6	39±5	Skin softened

The results given in Table 1 show that Phaltan at 5.0 μg/ml., used as a pre- or post-inoculation spray almost completely suppressed the rot without any adverse effects on the skin of the fruits. A pre-inoculation spray of Bordeaux mixture (4 : 4 : 50) gave an equally effective control of the disease. Both these fungicides may control the disease in the field. Spraying with cycloheximide resulted in softening of the fruit skin thus resulting in abundant *Penicillium* rot.

Phaltan, which has recently been shown to inhibit rot-inducing enzymes of other pathogens (Grover and Moore, 1962 ; Grover, 1964) and is also known to control brown rot of cherries (Grover, 1962), Brown rot of peaches (Cation, 1959), and other vegetable diseases too, appears to be particularly promising. Results of trials proposed to be undertaken in this connection will be reported later.

SUMMARY

Eight fungicides were initially tested *in vitro* against *Alternaria citri* Pierce, the causal agent of black rot of sweet orange. Copper sulphate, dodine, phaltan and cycloheximide were found to be extremely toxic. These along with Bordeaux mixture were then tested against the disease in the laboratory, and of all phaltan was found to be the most effective. Bordeaux mixture was a close second. It is suggested that these fungicides may control the disease in the field.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : Our thanks are due to Professor P. N. Mehra and Mr. C. S. Pracer for facilitating these investigations.

Regional Fruit Research Station,
Punjab Agricultural University,
Abohar (Punjab).
and
Botany Department,
Panjab University,
Chandigarh (Punjab).

REFERENCES

American Phytopathological Society, Committee on Standardization of Fungicidal Tests. (1947) The test-tube dilution technique for use with the slide germination method of evaluating protectant fungicides. *Phytopathology*, **37** : 354-356.

CATION, D. (1959) Some fungicide trials on tree fruits in 1958. *Quart. Bull. Mich. Agr. Exp. Sta.*, **41** : 685-698.

DIMOND, A. E., J. G., HORSFALL, J. W. HEUBERGER AND E. M. STODDARD, (1941) Role of dosage response curve in the evaluation of fungicides. *Bull. Conn. Agri. Expt. Sta.*, **451** : 635-667.

ECKERT, J. W. AND M. J. KOLBEZEN, (1963) Sym-dibromotetrachloroethane—a new fungistat for control of citrus fruit decay. *Phytopathology*, **53** : 755-761.

GROVER, R. K. (1962) Field evaluation of antibiotics and fungicides used for the control of blossom blight and ripe-fruit-rot of sour cherries caused by *Sclerotinia fructicola* and *S. laxa*. *Research Bull. (N. S.) Panjab Univ.*, **13** : 103-114.

GROVER, R. K. (1964) Effect of some fungicides on pectolytic enzyme activity of *Sclerotinia sclerotiorum* and *Botrytis allii*. *Phytopathology*, **54** : 130-133.

GROVER, R. K. AND J. D. MOORE, (1962) Toximetric studies of fungicides against brown rot organisms, *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, **52** : 876-880.

HORSFALL, J. G. (1956) *Principles of Fungicidal Action*, 279 pp. Chronica Botanica, Waltham, Mass.

UPPAL, B. N., M. K. PATEL, AND M. N. KAMAT, (1935) *The Fungi of Bombay*, 56 pp. *Bombay Dept. Agric. Bull.*, **176**.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

Chemical sprays for the control of Secondary Infection of Black Rot of Cauliflower :—Y. P. RAO AND S. B. MATHUR, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—Rao and Srivastava (unpublished data) have reported an outbreak in an epidemic form of the bacterial black rot disease incited by *Xanthomonas campestris* (Pammel) Dowson in the seed crop of cauliflower, variety *Snowball-16* at the Indian Agricultural Research Institute, Vegetable Breeding Substation, Katrain (Kulu). Although complete control of the disease is possible only by hot water treatment of the seed combined with other precautions, the disease at Katrain in most of the fields was so severe that chemical spraying had to be given in a bid to reduce damage to the crop by the disease. The general crop was sprayed with the fungicide Zineb (0.15 per cent). It was also considered desirable to test some other chemicals including *Agrimycin 100 on an experimental scale against the disease.

A crop of cauliflower, variety *Snowball-16* in an area of about 0.1 acre was selected and divided into five equal plots, the number of plants in the plots being 65, 77, 80, 63 and 69, respectively. A careful examination of the plants showed that the infection was nearly 100 per cent but it was confined to only the lowermost leaves. Before spraying, the infected leaves were removed from the plants. The first four plots were sprayed twice at an interval of 15 days during February and March, 1964 with Agrimycin 100 (0.02 per cent), Fytolan (Copper oxychloride) 0.15 per cent, Agrimycin and Fytolan in the above concentrations, Zineb (Zinc ethylene bisdithiocarbamate) 0.15 per cent, respectively, and the fifth plot was sprayed with plain water only to serve as "Control". Observations were recorded on 25th March, 1964, 10 days after the second spray. The efficacy of the various sprays was assessed by the number of plants forming normal curds. Thereafter the weather became dry and the disease did not develop further.

In the case of Agrimycin and Agrimycin+Fytolan, 23 and 25 normal curds were formed out of 65 and 80 plants, respectively. In Fytolan and Zineb, the number of plants that formed normal curds were 22 and 11 out of 77 and 63, respectively. In the Control, only 7 out of 69 plants formed normal curds.

The increase in the number of curds as a result of fungicidal protection was accompanied by an increase in the size of the curds. The results show that Agrimycin 100 which is known to be effective against species of *Xanthomonas* afforded protection against the disease. Use of the other fungicides had litttle effect. Unfortunately, Agrimycin 100 is not yet sold in this country. As the experiment was performed with a little "Free sample" available in the Division, large scale replicated trials could not be undertaken.

---

*A Charles Pfizer antibiotic preparation, containing 15 per cent Streptomycin. 1.5 per cent Oxytetracyclin and 83.5 per cent inert material.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Even though the seeds sown in a particular field may be disinfected or may be disease free, the chances of infection through infected plant refuse of the previous season or from a untreated crop from an adjacent field cannot be ruled out. The importance of spraying the crop with these chemicals lies essentially in preventing the secondary infection of the crop. It is felt that treatment of the seed combined with chemical sprays during the growing season could offer better protection to the crop.

Our grateful thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology and Dr. D. N. Srivastava, Plant Bacteriologist of this Division, for their useful suggestions during the course of this investigation. Thanks are also due to the Vegetable Specialist, Katrain (Kulu) Substation and his staff for kind cooperation in this work.

Control of Damping off of Cauliflower :—H. S. SOHI, S. L. SHARMA, K. B. SACHDEVA AND B. R. VERMA, Section of Plant Pathology, Department of Agriculture, Himachal Pradesh, Solan—Upto 80 per cent losses of cauliflower seedlings are caused by damping-off at the Government Vegetable Research Station, Solan in Himachal Pradesh. *Rhizoctonia solani* Kühn was found to be associated with the disease in 90 per cent of the cases. Burkett[1] reported effective control of damping-off of cauliflower, cabbage, etc. with Ceresan dust and Copper carbonate seed treatment, while Campbell[2] found soil application of Pentachloronitrobenzene and Mercuric chloride solution to give good control of damping-off due to *R. solani* Kühn. No work, however, seems to have been done in India so far on this disease. Some common fungicides, *viz.*, Agrosan GN, Thiram wet, Tillex, Copper carbonate, Flit 406 and Cheshunt compound were, therefore, tried in these studies. Ceresan dry, PCNB and Mercuric chloride were also included in the tests for comparison.

Mercuric chloride (0.1 per cent aqueous solution) and Cheshunt compound (1.25 per cent aqueous solution) were tried as soil drench at the rate of 1.5 litres/7 kg. of soil ; the rest of the fungicides were applied as seed-dressings at full dose, removing the extra fungicide by sieving after the treatment. The experiments were conducted on two occasions with seeds of var. Snowball, in artificially inoculated soil in pots. Observations were taken on both pre and post-emergence mortality of seedlings, which are given in table 1.

It is evident that none of the treatments brought about a complete control of either phase of the disease. Seed treatment with Ceresan dry and Mercuric chloride treatment of soil gave almost equally effective control in both the tests, the total average losses being 34.5 and 33.0 per cent respectively, as against 87 per cent in the control. Other treatments were much less effective. Cheshunt compound was toxic to root formation and whatever seeds germinated collapsed within 3 to 4 days as the roots did not develop. It was further observed that soil treatment with mercuric chloride slightly delayed germination. Since soil treatment with mercuric chloride is, obviously cumbersome, dressing the seed with

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1

S. No.	Treatment	Expt. conducted on 21.11.63			Expt. conducted on 24.8.64			
		Per cent pre-emergence loss	Per cent post-emergence loss	Per cent Total loss	Per cent pre-emergence loss	Per cent post-emergence loss	Per cent Total loss	Average total loss
1.	Agrosan GN	13	6	18	39	55	73	45.5
2.	Ceresan dry	18	5	23	34	18	46	34.5
3.	Thiram wet	19	23	38	12	42	48	43.0
4.	Tillex	25	12	34	52	91	96	65.0
5.	Copper carbonate	26	19	40	22	63	71	55.5
6.	PCNB 75 per cent dust	11	7	17	12	50	56	36.5
7.	Flit 406	27	17	39	31	83	88	63.5
8.	Mercuric chloride	19	13	30	26	13	36	33.0
9.	Cheshunt compound	56	100	100	...	...	...	100.00
10.	Control	79	19	83	36	86	91	87.0

Ceresan dry appears to be a promising method of reducing damping-off of cauliflower seedlings under our conditions as well.

ACKNOWLEDGEMENTS : The authors are greatly indebted to Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh, for his keen interest in these studies. Thanks are also due to Shri S. L. Juneja, Vegetable Botanist, Himachal Pradesh, for supplying the cauliflower seeds for the experiments.

*[1]Burkett A. L. *Rep. Texas agric. Exp. Stn.*, pp. 72-81 (1938).
[2]Campbell L. *Plant Dis. Reptr.*, **38** : 859 (1954).

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Identification of a new biotype within Indian race 162 of Puccinia recondita Rob. ex Desm.—R. PRASADA, S. K. SHARMA AND M. M. MERCHANDA. Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12.—Success of any wheat improvement programme with respect to resistance to rusts depends very largely upon a sound knowledge of physiologic races prevalent in the country, because it is with the help of these races that the wheat breeder discovers sources of resistance and subsequently screens the progeny of such crosses that he may make with agronomically desirable parents. Several investigators have, however, reported that these physiologic races, as indentified with the help of standard differential hosts, can be further subdivided into biotypes if additional host varieties are included in pathogenicity tests (Stakman, 1954 and Prasada and Sreekantiah, 1956). These biotypes are generally more virulent than the races themselves and, for that reason, their identification is of great practical importance in wheat improvement programme.

In India several biotypes have been recorded in wheat rusts by different workers. In the present paper, the occurrence of a virulent biotype of race 162 of *Puccinia recondita* and its distinguishing characters have been reported.

Race 162 of *P. recondita* was first isolated in India from a rust collection of 1955-56 crop from Gwalior (M. P.) on wheat variety N. P. 720 by Misra *et al.* (1960). From 0.56 per cent in 1955-56, the prevalence of this race increased to 43.5 per cent in 1962-63 and it came to be considered an important race. Besides its wide prevalence, the host range of this race had a great impact on the breeding programme. In early breeding work, Mediterranean (E. 67) and Democrat (E. 66) which were resistant to the known Indian races, had been widely used as sources of resistance but, since both these varieties are susceptible to race 162 most of the progeny derived from these crosses failed to stand up to this race.

For the identification of physiologic races of *P. recondita*, a set of 8 standard differential hosts originally selected by Johnston and Mains (1932) has been used here as in almost every other country. Race 162 was identified with the help of these differential hosts. A biotype of this race was isolated from a 1959-60 crop collection from Rithani (U. P.) to which wheat variety "Thew" was susceptible whereas it was resistant to the type race 162 isolated in 1955-56 from Gwalior. This biotype has been labelled 162-A on account of its virulence on Thew (Payak : unpublished).

In the present studies, collections of race 162, identified in 4 different rust samples obtained from widely separated localities Kalimpong (Bengal), Gurdaspur (Punjab), Kalyani (Bengal) and Bareilly (U. P.), were studied on a large number of wheat varieties and their reaction types compared with the type cultures of race 162 and biotype 162-A. There was no difference in the reaction of differential hosts but differences were observed on wheat Henry (E. 790) as shown in the Table 1.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Reaction of variety "Henry" against type race 162 and its new biotype.

S. No.	Race	Place of collection	Original Host	Henry (C. 1.122.65) (E. 790)
1.	162 Type Race	Gwalior	N. P. 720	0 ;-1
2.	162	*Kalimpong	Ridley	4
3.	162	Kalyani	N. P. 87	4
4.	162	Gurdaspur	E. 537	4
5.	162	Bareilly	Local	4

*Single spore culture of this isolate has been established and is being maintained as a Type Culture to represent biotype 162-B.

From the above data, it is apparent that there are differences in pathogenicity on Henry between Type race of 162 and the biotype (designated 162-B) reported herein. Compared with biotype 162-A, Thew is susceptible to 162-A but resistant to 162-B. Differences were observed on some other varieties also and these have been supplied in Table 2.

TABLE 2 : Differences between race 162 and its biotypes (A and B) on some selected varieties

Race	E. 5370	Thew	E. 5982	Henry
162	0 ;	0 ;—1	4	0 ;—1
162—A	0 ;	4	4	0 ;—1
162—B	4	0 ;—1	0 ;	4

Grateful thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for providing necessary facilities and taking helpful interest.

Johnston, C. O. and E. B. Mains. Studies on physiologic specialisation in *Puccinia triticina*. Dept. of Agric. Tech. Bull. 313 (1932).

Misra, D. P., V. C. Lele and Vasudeva, R. S. Race 162 of brown rust of wheat. A new record for India. Ind. Phytopath., **13** : 183 (1960).

Prasada, R. and K. R. Sreekantiah. Identification of Biotypes within Physiologic Races of Wheat Stem Rust. *Indian Phytopath.*, **9** : 209-211 (1956).

Stakman, E. C. Recent studies of wheat stem rust in relation to breeding resistant varieties. Phytopathology., 44 (7) pp. 346-351 (1954).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Helminthosporium carbonum Ullstrup, on maize in India—

T. P. BHOWMIK AND B. L. CHONA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12.

Heminthosporium leaf blight is the most destructive disease of maize in India that limits the successful cultivation of the crop every year. Out of the three species that are commonly involved in causing the leaf blight, namely, *Helminthosporium turcicum* Pass., *H. maydis* Nishik. and Miy., and *H. carbonum* Ullstrup, only the first two species were known so far to be present in India. Though the disease is widespread, it is particularly more severe in the Southern parts, Terai regions and the sub-Himalayan ranges of Northern India. In the cool humid regions of the country, *H. turcicum* is the principal pathogen in causing the blight disease, while in comparatively warmer regions, leaf spot due to *H. maydis* predominates. The present paper records the presence of *H. carbonum* Ullstrup on maize for the first time in India.

Ullstrup[1] described a new species *Helminthosporium carbonum* causing leaf spot on maize from the United States. He recognised two races of the pathogen on the basis of symptoms produced although they are morphologically indistinguishable. In case of race-1 of the pathogen, the lesions are small having light brown centre and purple brown margins with zonate pattern while in case of race-2, the spots are irregular, chocolate brown in colour without any zonations.

Leaf samples collected in September 1963 from Gangtok, Sikkim State (Altitude 5500 ft.) showed the presence of spots which were distinctly different from those caused by either *H. turcicum* or *H. maydis*. The spots were small, round to irregular in shape and light brown in colour. Isolations from these spots invariably showed the presence of a *Helminthosporium* sp. which was different from both *H. turcicum* and *H. maydis* in respect of conidial morphology. Monoconidial culture of the pathogen was, therefore, established and multiplied on Potato-Dextrose-Agar slants by the usual method.

Young maize seedlings became readily infected when water suspension of conidia was sprayed on them under conditions of optimum temperature and humidity. To study the morphological character of the pathogen, dried infected leaf material was incubated in moist chambers at 25°C for 4-6 days and this resulted in heavy sporulation. Measurements of conidia and conidiophores gave the following results:—

Conidia are dark olivaceous brown, straight or sometime slightly curved, cigar-shaped, widest in the middle and tapering slightly toward the rounded ends, measure 26-92 × 9-16 microns (mean = 58 × 12 microns), are 3—9 septate (mean = 6), have an inconspicuous hilum and germinate by two polar cells. Conidiophores normally arise singly or in groups of two or more through the stomata. They are dark olivaceous brown in colour, sometimes branched, geniculate and measure 52-408 × 4-7 microns (mean = 189 × 6 microns) (Fig. 1). According to Ullstrup[1], the conidia of *H. carbonum* measure 25-100 × 7-18 microns (mean = 62.6 × 13.2 microns), 2-12 septate (mean = 7) and the measurements of conidiophores vary from 90-230 × 5-7 microns. Since the shape, colour and measurements

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

of both conidia and conidiophores are very close to that given by Ullstrup[1], the fungus is identified as *Helminthosporium carbonum* Ullstrup. Further work with this pathogen is in progress and will be reported in due course.

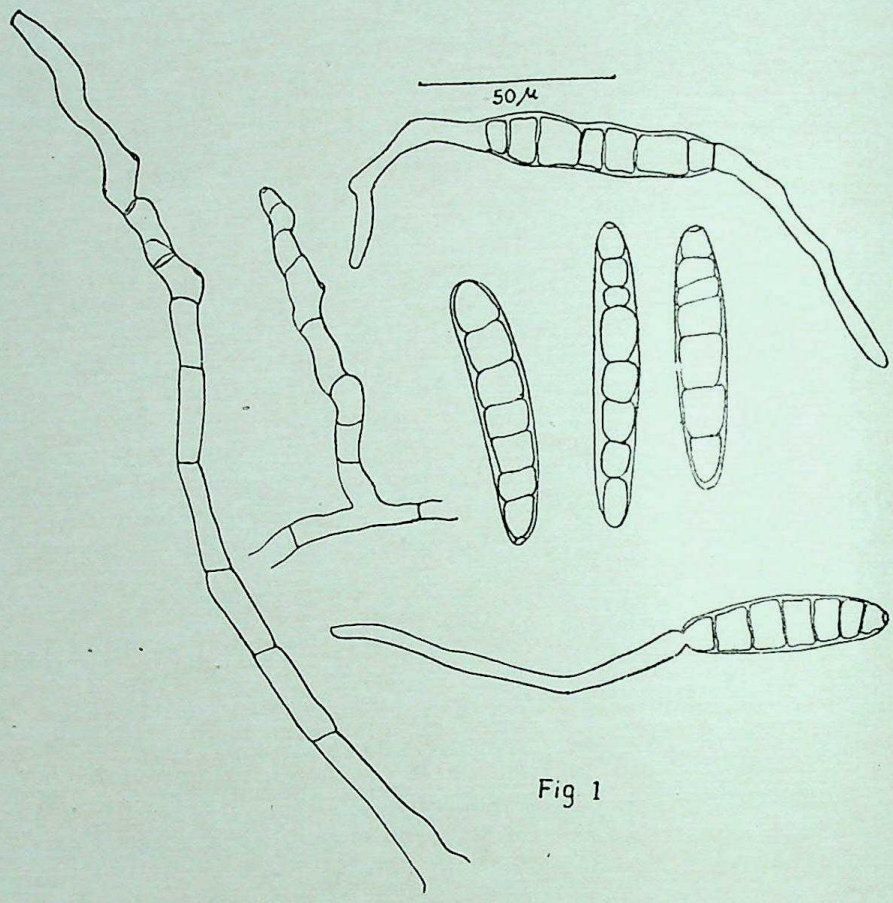


Fig 1

ACKNOWLEDGEMENTS: The authors are grateful to Mr. R. L. Munjal, Systematic Mycologist, Division of Mycology and Plant Pathology for help in identification of the fungus. The authors also express their grateful thanks to Dr. B. L. Renfro, Plant Pathologist, Rockefeller Foundation, Co-ordinated maize Improvement Scheme for the supply of the diseased maize leaf samples.

[1]Ullstrup, A. J., *Phytopathology*, 34 : 214-222, (1944).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SEVENTEENTH ANNUAL REPORT OF THE INDIAN PHYTOPATHOLOGICAL SOCIETY, 1963

Mr. President, members of the I. P. S., Ladies and Gentlemen,

I am presenting herewith the seventeenth annual report of the Society for the year 1963. Fifty three new members joined the Society, 19 as life-members and 34 as ordinary members. It is regretted that the membership of 47 members had to be discontinued due to non-payment of their dues and having no response from them in spite of several reminders sent to them. The total number of the members at the end of the year was 311 and that of subscribers 213. Now the subscription is due from a few members only.

During the year, volume XVI, numbers 1, 2 and 3, 1963 of *Indian Phytopathology* have been issued. The financial position of the Society continued to be satisfactory. Besides, a sum of Rs. 7,500 continues to remain in the form of National Savings Certificate. The amount to the credit of the Society in the savings bank account with M/s National and Grindlays Bank Ltd. (Lloyds Branch), New Delhi and cash in hand taken together on 31.12.1963, was Rs. 18,992.13. Thus the assets of the Society amount to Rs. 26,492.13. In addition, several cheques were in the process of collection at the end of the year under report.

During the year, the receipts amounted to Rs. 17,139.89 and expenditure to Rs. 12,346.89, thus showing a balance of Rs. 4,793.00 in favour of the Society. It may be mentioned that no financial assistance was received by the Society from any source during the year. We have paid the cost of printing vol. XV, Nos. 2, 3 and 4, of 1962. The printing of the journal was in the hands of M/s. Oxford Printing Works, New Delhi.

During the year under report, two council meetings were held to discuss the business of the Society.

Two important points discussed in detail during the Council Meetings were (i) Arranging an International Symposium on Recent advances in research on 'Diseases of Tropical Plants' and (ii) Proposed project on 'Manual of plant diseases in India'. Dr. P. R. Mehta and Prof. T. S. Sadasivan were primarily responsible for these ideas and we are very thankful to them. Thirteen papers read in the symposium on 'Role of therapeutic treatments for control of plant diseases' in February 1963, were published in Bulletin No. 1 of the Society.

The following lectures by eminent scientists were arranged during the year 1964:—

1. 2-1-1963. Dr. D. J. Raski, Visiting Professor from University of California 'Are nematodes plant pathogens ?'

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

2. 23-8-1963. Dr. W. G. Solheim, Acting Dean and Professor of Botany, Wyoming University, U. S. A. "Vegetational Zonation of Wyoming with special reference to plant diseases".

3. 14-9-1963. Dr. Karl Maramorosch of Boyce Thompson Institute for Plant Research, New York, U. S. A. "Multiplication of plant pathogenic viruses in insects".

Before I close, I may mention that we are indeed thankful to Prof. C. V. Subramanian, for organizing the symposium on 'Host Parasite relationships in plant diseases". I am most thankful to Prof. T. S. Sadasivan, President of the Society, Dr. P. R. Mehta, the immediate Past President and Dr. R. Prasada, the Chief Editor for giving valuable suggestions from time to time. Lastly, on behalf of the Society, I express our sincere thanks to the Indian Science Congress Association and the University College of Science, Calcutta, for providing necessary facilities to conduct the meeting in the Science College Building at Calcutta. I am also thankful to Dr. D. Suryanarayana, Joint Secretary of the Society, and Dr. M. M. Payak, member of the Editorial Board for helping me in various ways in carrying out my work as Secretary-Treasurer during the year under report.

Sd/- (S. P. Raychaudhuri)

Dated : December 29, 1964. *Secretary-Treasurer*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Abstracts of Papers read in the Symposium on "Physiology of Host-parasite relationships in Plant Diseases" during the Seventeenth Annual General Meeting of the Indian Phytopathological Society held at Calcutta, on 29th December, 1964.

Host Parsite Relationship in Rusts. R. Prasada, Indian Agricultural Research Institute New Delhi.—Rusts are obligate parasites. Host-parasite relationship in their case commences from the time nutritional connection is established between the two. The germ-tube may enter a non-congenial host wirhout making any further progress but this cannot be considered infection. Rusts are those parasites that obtain their nutrients from living cells, often without killing them at all or at least not for a long time. Age of the host and environment, particularly temperature and humidity, are important factors. In a susceptible host, the rust pathogen produces very little or no hypersensitive reaction while in resistant hosts either there is no reaction visible or the host cells are killed which restricts further growth of the parasite. There are various gradations between the two.

In vitro growth of insect induced plants galls. H. C. Arya, Department of Botany, University of Rajasthan, Jaipur.—Observations are recorded on a variety of insect galls in plants. Detailed studies in tissue culture were made of gall tissue incited by *Phylloxera vastatrix* Planch. Isolations of the gall and normal grape stem tissues were made. A large number of single-cell isolations were obtained out of the callus formed following nurse tissue technique. Six single-cell clones, 3 from the normal tissue, and 3 from the gall tissue were studied. Tissue clones were grown on modified White's basal medium supplemented with pancreatic digest of casein hydrolysate and other nutritive ingredients. The cultures were grown in the dark at 26°C and 55 per cent relative humidity. During the growth of the tissues, four harvests were taken at an interval of 10 days for 40 days. The oven-dried tissues at 65°C were used as tissue samples.

Effect of alpha-naphthaleneacetic acid (NAA) and nucleic acids (RNA and DNA) on the nucleic acid contents of the gall and normal single-cell clones were studied. The nucleic acid fractions were obtained with the chemical extraction method. The amounts of RNA and DNA were determined with the help of 'DU' spectrophotometer.

RNA was in direct relation with the growth of the gall and normal tissue clones. RNA contents of the tissues were directly impressed with the external application of NAA. The RNA content of the normal tissue clones rose remarkably at 1.0 mg/litre of NAA in the nutritive medium. However, addition of NAA did not get any appreciable response from the gall tissue clones.

By increasing the concentration of RNA in the medium, the RNA contents of the normal clones increased upto a certain level of its

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

concentration (40 mg/litre). However, further increase of RNA in medium (400 mg/litre) reduced the RNA contents of both the groups of tissue. Effect of DNA in the medium was important since the decrease in the DNA content of the gall clones was stopped and the curves became steadily like those of the normal clones. The results established the important role of the nucleic acids in the disease phenomenon.

Some Aspects in Virus Infection of Plants. R. S. Badami, University Botany Laboratory, Madras.-5.—Plant viruses have unique ability to thrive only in the living cells of host plants. As such, in response to virus infections, several avenues of reactions occur in the host cells of virus-infected plants. An important and yet very characteristic and specific feature is the notable cytological changes that follow early in virus infections of host plants. There is production of various types of intracellular inclusion bodies : amoeboid, crystalline and intranuclear inclusions. These are essentially absent in healthy, host plants or in plants infected with any disease other than a virus, thus making this simple yet effective diagnostic feature of virus infections. Concurrent with appearance of external symptoms in virus infection, formation of inclusion bodies is noticed abundantly in hair and epidermal cells but not in apical meristem. Their production depends more upon the infecting virus than upon the host plant infected, although the host plant does play a part in formation of inclusions.

In nearly over forty or more (about 10 per cent of plant virus diseases known) plant virus diseases production of inclusion bodies has been noted, but these are not necessarily formed in all virus diseases. They are formed in association with the nucleus and are infective, protein in nature. These can be recognised by strain reactions. Both light and electron microscopy are increasingly applied to study these curious bodies in much greater detail to elucidate their structure.

These are not the breakdown products of reaction products of cells to the disease. But the formation of the inclusions possibly in part acts as a protective mechanism.

Plant tissue culture—a tool for studying host virus relationships. M. D. Mishra, S. P. Raychaudhuri and H. C. Phatak, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi 12.—The development of the tissue culture technique has opened new lines of work in experimental biology. Some of the basic problems of pathogenicity especially those concerned with the intimate relationship of host and parasite at the cellular level have been tackled efficiently. Slower pace in the progress of plant virus research may be due to the fact that only the meristems from the plants can be multiplied on a suitable medium and it is comparatively easy to infect animal cells and tissues in culture and to study virus multiplication in them. Three types of tissues, namely normal, conditioned and tumorous, are employed for plant tissue culture studies. Media proposed by White in 1934, Gautheret, Hildebrandt and others are used as such or in modified forms. Morel has done work especially with tissue cultures of tumorous origin.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The authors have been successful in cultivating chilli mosaic virus (ChMV), Sunn-hemp mosaic virus (SMV), tobacco mosaic virus (TMV) and a ring spot strain of potato virus X (PVX). They have studied the effect on the viral multiplication by the addition of purine and pyrimidine bases of the nucleic acid to the medium. By employing *Chenopodium amaranticolor* Coste and Reyn. as the local lesion host in case of PVX and ChMV they found that the nucleic acid bases appreciably increased the virus content of the cultured tissue. They also found that yeast RNA had no effect on the virus content.

The most important utilization of the tissue culture technique is to obtain virus-free shoot-tips of the crops of agri-horticultural interest, by taking advantage of the slower rate of the movement of the virus as compared to the fast-growing shoots.

Pathogens in the rhizosphere. N. G. Nair, Department of Botany University of Rajasthan, Jaipur.—The significance of critical studies on the autecology of soil-borne pathogens in the rhizosphere is indicated. Past investigations on the subject are briefly reviewed. Detailed consideration is given to the behaviour of *Bipolaris sorokiniana* (*Helminthosporium sativum*) in the rhizosphere of wheat plants with special reference to certain host factors, especially ploidy and root exudate patterns and soil factors. The results are discussed critically *vis-a-vis* the foot rot problem.

Plant nematode relationship. Kishan Singh, Indian Institute of Sugarcane Research, Lucknow.—The relationship of plant nematodes with their hosts can be of a parasite, cooperator, coagent and/or of a vector.

For specialized plant parasitism, the essential requirements for nematodes appear to be endoparasitism and rapid reproduction without jeopardising the host physiology too much so as to maintain a continuous food supply. The root knot nematode, *Meliodogyne* spp., which fits well within these limitations does not cause necrosis of the attacked cells and is highly evolved for parasitism, morphologically as well as physiologically. Most of the plant parasitic nematodes cause various degrees of hypertrophy with or without hyperplasia. This is most pronounced in the case of *Meloidogyne*. Production of an excess of indole-acetic acid from tryptophane, a product of the action of pectolytic enzymes contained in the saliva are possibly responsible for the growth of isodiametric cells resulting into galls in case of root knot nematodes and swellings in several others. Incitation of hyperplasia in the meristematic cells and tumors in the pericycle of the host by *Radipholus similis*, the burrowing nematode, is another example of hormone like activity in the parasitised rootlets. In many ways, the response of root cells to nematode parasitisation is similar to reactions on application of growth regulating substances. Action of *Trichodorus christiei*, an ectoparasite, which feeds just behind root caps is rather peculiar. It causes a slight hypertrophy of the rootlets on the one hand and suppresses cell division on the other. Though the injury appears to be slight, this 'devitalisation' of roots results in a marked host injury which in maize becomes apparent within a matter of days. Production of chitinase, cellulase, amylase and protopectinase has been found in *Ditylenchus* spp. In the case of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Radopholus similis, though the mechanism is not clearly understood, its movement within the host tissues and the formation of tunnels and cavities appears to be due to dissolution of cell walls by the enzymes secreted with saliva.

In wilt of cotton and root rot of tobacco and sugarcane, nematodes are associated as cooperators; in cauliflower disease of strawberry and the yellow slime disease of wheat they act as coagents; and in the fan leaf disease of grape vine nematodes are associated as virus vectors. These are some of the examples where nematodes act in complementary roles with other plant pathogens. A nematode-Pythium complex was recently found to be the cause of high incidence of plant mortality in sugarcane at Lucknow. It has also been seen that mineral deficiencies that may not even be noticeable in soils, are quite apparent where plants in such soils are parasitised by nematodes.

Metabolites of Helminthosporinm spp. in relation to plant disease. P. D. Tyagi, Department of Botany, University of Rajasthan, Jaipur.—Metabolites of *Helminthosporium* spp. implicated in disease include toxins produced by *H. oryzae*, *H. victoriae* and *H. sativum*. Of these, the *victoriae* toxin which has been studied somewhat in detail is unique in being specific to susceptible oat varieties, an example of positive correlation between toxigenicity and pathogenicity. Detailed consideration is given to the results obtained by the author on metabolites of *H. sativum* and the effects of some factors on toxin production *in vitro* are mentioned. On the basis of the results obtained, the role of toxins in the foot rot disease of wheat is discussed.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Indian Phytopathology

VOLUME XVII, 1964

Consisting of viii + 344 Pages

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

No. 1 JANUARY-MARCH

PAGES

In memorium Dr. Rama Rao Sridhar Badami—*T. S. Sadasivan* 1

Central Himalayan Fungi—I—*S. K. Bose and Emil Muller* ... 3

Studies on four isolates of Rhizoctonia solani Kuehn., the causai agent of black scurf disease of Potato in the Punjab—I—*Madho Singh* 23

On the Physiology of Ascospore formation in powdery mildew of wheat—*H. C. Arya* 27

Adaptation of Sclerotium rolfsii to Copper compounds—*D. V. Raghava Reddy and A. Apparao*... 35

New records of Plant Diseases from Himachal Pradesh—*H. S. Sohi, S. S. Jain, S. L. Sharma and B. R. Verma* ... 42

Furrow application of fungicides for control of Cotton seedling damping-off in Israel—*Z. Solel and G. Minz* ... 46

Fusarium seedling blight of Maize in Rajasthan—*H. C. Arya and B. L. Jain* 51

Further studies on foliar necrosis of Potato—*B. Ganguly and S. P. Raychaudhuri* 58

Experiments on Physiologic Specialization in Puccinia penniseti Zimm.—*G. G. Dalela and S. Sinha* ... 63

Ascomycetes on Celastrus paniculata Willd. II—*S. T. Tilak* ... 66

Phytopathological Notes

A note on the prevalence of Meloidogyne spp. in vegetable crops—*C. L. Sethi, J. S. Gill and G. Swarup* ... 69

A new Sorosporium species from Assam—*A. K. Roy* ... 71

A new Physiologic race of Puccinia striiformis West. in India—*S. K. Sharma and Sheodhan Singh* 72

Lipolytic activity of some Xanthomonads—*A. [illegible]. Padhya, W. V. Kotasthane and M. K. Patel* 73

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Occurrence of Periconia sacchari Johnst. and Phyllosticta sacchari Speg. on Sugarcane in Assam—*A. K. Roy* ... 75

Controlof leaf spot disease of spinach caused by Cercospora beticola—*R. L. Mathur and S. P. Sehgal* 75

Seed transmission and epidemiology of the bacterial blight disease of rice in North India—*D. N. Srivastava and Y. P. Rao* 77

Manuscripts for Indian Phytopathology 79

NO. 2 APRIL-JUNE

Study on eleven isolates of Phyllosticta rabiei (Pass.) Trot., the causal agent of Gram blight in the Punjab—*Shamsher Singh Aujla* 83

Root-knot of vegetables III. Factors affecting hatching of eggs of Meloidogyne javanica javanica (Treub, 1885) Chitw., 1949 and M. incognita incognita (Kofoid and White, 1919) Chitw., 1949—*Gopal Swarup and K. J. Pillai* 88

Factors influencing larval hatching in the root-knot nematodes, Meloidogyne incognita (Kofoid and White., 1919), Chitwood, 1949, and M. javanica (Treub. 1885), Chitwood, 1949—I-EFFECT OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION—*Asad Ahmed and Abrar M. Khan* 98

Factors influencing larval hatching in the root-knot nematode Meloidogyne incognita (Kofoid and White., 1919), Chitwood, 1949—II-EFFECT OF ROOT LEACHATES AND CERTAIN CHEMICALS—*Asad Ahmed and Abrar M. Khan* 102

Environmental conditions and false smut incidence in rice—*K. Manibhushan Rao* 110

Effects of varying water supply on the susceptibility on Gomphrena globosa L. plants to infection with potato virus X—*D. C. Sharma* 115

Effect of some vitamins on the growth of Fusarium oxysporum F. cumini isolates—*B. L. Mathur, L. C. Sharma and N. Prasad* 121

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Studies on host range and histopathology of root-knot infections caused by Meloidogyne javanica—*M. Balasubramanian and G. Rangaswami* 126

Some observations on linseed wilt—*L. B. Goel and Gopal Swarup* 133

Natural infection of Oxalis corniculata L., the alternate host of Puccinia sorghi Schw., in India—*D. P. Misra and S. K. Sharma* 138

Further studies in the crown rust of oat in India—*D. P. Misra, S. K. Sharma, P. C. Joshi and Sheodhan Singh* ... 142

Ramularia areola Atk. on herbaceum cottons in Vidharbha (Maharashtra)—*M. R. Siddiqui and S. B. P. Rao* ... 146

Three new bacterial diseases of plants from Maharashtra—*L. Moniz and V. P. Bhide* 149

Evaluation of certain fungicides for the control of Rhizoctonia bataticola (Taub.) Butler and Sclerotium rolfsii Sacc.—*Dharam Vir, R. D. Pathak and S. B. Mathur* ... 154

Effect of spore age and culture media on the degree of infection by Ustilago avenae—*Avdhesh Narain* 157

Efficacy of different fungicides-VIII : Field trials for the control of covered smut of barley [Ustilago hordei) (Pers.) Lager.]—*J. S. Grewal and Dharam Vir* ... 162

Sorodiscus radicicolus Cook, a new record for India—*M. S. Ghemawat* 165

Pathogenicity and varietal reaction of paddy to Sclerotium oryzae Catt. in Bihar—*A. P. Misra and Abu Mohammad* 168

Seed rot of Hevea brasiliensis caused by Botryodiplodia theobromae Pat.—*S. N. S. Srivastava* 172

Phytopathological Notes

A New Stilbospora species from India—*M. Shukla* 178

Occurrence of Stemgall disease of jute caused by Physoderma corchori Lingappa in W. Bengal—*C. R. Das and P. K. Sen Gupta* 180

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Some fern-inhabiting Cercospora species from India—*G. Lall, J. N. Kapoor and R. L. Munjal* 181

Maintenance of fungi on synthetic sponge—*N. S. Subba Rao* ... 183

Review 184

NO. 3 JULY-SEPTEMBER

Dr. Jonas J. Christensen 186

A new leaf spot disease of Jowar in India—*Mahendra Pal and D. Suryanarayana* 188

Studies in Pectic enzymes of parasitic fungi—V. PECTIC ENZYMES PRODUCED BY PENICILLIUM EXPANSUM LINK—*S. C. Gupta and G. S. Rautela* 191

Studies on the survival of plant pathogens added to the soil—III. ON FOUR PHYTOPATHOGENIC BACTERIAL SPECIES—*G. Rangaswami and V. Thirunavukarasu* 202

A preliminary list of microfungi associated with the decaying stems of Triticum vulgare L. and Andropogon sorghum L.—*S. P. Lal and A. S. Yadav* 208

Studies on the population of Heterodera avenae Woollen. causing Molya disease of wheat and barley in Rajasthan—*Kishan Singh and Gopal Swarup* 212

Pycnidial stage of Hendersonula toruloidea on twigs of Psidium guajava in artificial culture—*R. L. Mathur and R. D. Singh* 216

Induced saltations in Helminthosporium oryzae Breda de Haan—*A. P. Misra and A. K. Chatterjee* 218

The myxomycetes of Calcutta suburbs—*Asok Kumar Kar* ... 222

Enzymic studies of certain wood destroying fungi—*D. K. Saha* ... 224

Fungal microflora of seeds of Jowar (Sorghum vulgare) its role in reduced emergence and vigour of seedling and control—*R. L. Mathur and S. P. Sehgal* ... 227

Presence of amino acids and Indole compounds in the root-knot affected plants—*G. Rangaswami and M. Balasubramanian* 234

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Preliminary screening of wheat varieties against loose smut by 'Embryo test'—*R. L. Munjal and M. S. Chatrath* ... 238

Perithecial production by different strains of Glomerella tucumanensis (Speg.) Arx and Mueller—*B. L. Chona, B. S. Bajaj and Rajeshwari Sharma* 241

Role of Vulpia myuros and Briza minor in the perpetuation of black rust of oats in the Nilgiri Hills—*L. M. Joshi and V. C. Lele* 245

Observations on Indian aquatic fungi—II. OCCURRENCE OF OLPIDIOPSIS VARIANS SHANOR AT GORAKHPUR—*G. C. Srivastava* 249

Phytopathological Notes

A note on estimation of losses due to leaf blight of wheat caused by Alternaria triticina—*V. V. Chenulu and Amar Singh* 254

A new bacterial Canker of Carissa congesta in Maharashtra —*L. Moniz, J. E. Sabley and W. D. More* 256

Formation of secondary sporidia in Uromyces decoratus Syd.—*L. M. Joshi* 257

A Fusarium sp. mycoparasitic on stem rust of wheat, Puccinia graminis var. tritici (Pers.) Eriks and Henn.—*R. Prasada and S. K. Sharma* 258

Announcement 262

No. 4 OCTOBER-NOVEMBER

Respiration in plants under Pathogensis—*T. S. Sadasivan* ... 263

Further studies on Phytophthora blight of Sesamum—*P. D. Gemawat and N. Prasad* 273

Some Physiological studies on Sugarcane smut (Ustilago Scitaminea Syd.)—*P. Appalanarasayya* 284

The nature of resistance of paddy to Helminthosporium oryzae Breda De Haan—*A. P. Mishra and Y. Prasad* ... 287

Mutation in colletotrichum falcatum Went. the causal organism of red rot of Sugarcane—IV. MORPHOLOGICAL AND BIOCHEMICAL MUTANTS INDUCED BY IONIZING RADIATION—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* 296

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

A new pseudotorula from Rajasthan—*P. N. Mathur and G. Singh* 304

Studies on "Udbatta" disease of Rice—*N. N. Mohanty* ... 308

Some interesting fleshy Fungi from Himachal Pradesh—*H. S. Sohi, S. Kumar and P. K. Seth* 317

Occurrence of Tobacco mild motle virus on Tobacco—*H. N. Verma* 323

Evaluation of fungicides for the control of black rot of sweet orange—*Sushil Kumar and Rajendra K. Grover* ... 328

Phytopathological Notes

Chemical sprays for the control of secondary infection of black rot of cauliflower—*Y. P. Rao ond S. B. Mathur* ... 332

Control of damping-off of cauliflower—*H. S. Sohi, S. L. Sharma, K. B. Sachdeva and B. R. Verma* 333

Identification of new biotype within Indian race 162 of Puccinia recondita Rob ex Desm—*R. Prasada, S. K. Sharma and M. M. Merchanda* 335

Helminthosporium carbonum Ullstrup, on maize in India—*T. P. Bhowmik and B. L. Chona* 337

Seventeenth Annual Report of the Indian Phytopathological Society, 1963 339

Abstracts of papers read in the Symposium on "Physiology of Host-parasite relationships in plant diseases" during the Seventeenth Annual General Meeting of the Indian Phytopathological Society held at Calcutta, on 29th December, 1964 341

Index to volume XVII .. .. i—viii

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDEX TO AUTHORS

Ahmed, A. ... 98, 102
Appalanarasayya, P. ... 284
Apparao, A. ... 35
Arya, H. C. ... 27, 51
Aujla, S. S. ... 83
Bajaj, B. S. ... 241, 296
Balasubramanian, M. 126, 234
Bhide, V. P. ... 149
Bhowmik, T. P. ... 337
Bose, S. K. ... 3
Chatrath, M. S. ... 238, 296
Chatterjee, A. K. ... 218
Chenulu, V. V. ... 254
Chona, B. L. ... 241, 337
Dalela, G. G. ... 63
Das, C. R. ... 180
Dharam Vir ... 154, 162
Ganguly, B. ... 58
Gemawat, P. D. ... 273
Ghemawat, M. S. 165
Gill, J. S. ... 69
Goel, L. B. ... 133
Grewal, J. S. ... 162
Grover, R. K. ... 328
Gupta, S. C. 191
Jain, B. L. ... 51
Jain, S. S. ... 42
Joshi, L. M. ... 245, 257
Joshi, P. C. ... 142
Kapoor, J. N. ... 181
Kar, A. K. ... 222
Khan, A. M. ... 98, 102
Kotasthane, W. V. ... 73
Kumar, S. ... 317
Kumar Sushil ... 328
Lal, S. P. ... 208
Lall, G. ... 181
Lele, V. C. ... 245
Mathur, B. L. ... 121
Mathur, P. N. ... 304
Mathur R. L. ... 75, 216, 227
Mathur, S. B. ... 154, 332
Merchanda, M. M. ... 335
Minz, G. ... 46
Misra, A. P. ... 168, 218, 287
Misra, D. P. ... 138, 142
Mohammad, A. ... 168
Mohanty, N. N. ... 308
Moniz, L. ... 149, 256
More, W. D. ... 256
Müller, E. ... 3
Munjal, R. L. ... 181, 238
Narain, A. ... 157
Padhya, A. C. ... 73
Pal, M. ... 188
Patel, M. K. ... 73
Pathak, K. D. ... 154
Pillai, K. J. ... 88
Prasad, N. ... 121, 273
Prasad, Y. ... 287
Prasada, R. ... 258, 335
Rangaswami, G. ... 126, 202, 234
Rao, K. M. ... 110
Rao, S. B. P. ... 146
Rao, Y. P. ... 77, 332
Rautela, G. S. ... 191
Raychauduri, S. P. ... 58
Reddy, P. V. R. ... 35
Roy, A. K. ... 71, 75
Sabley, J. E. ... 256

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Sachdeva, K. B. ... 333
Sadasivan, T. S. ... 1, 262
Saha, D. K. ... 224
Sehgal, S. P. ... 75, 227
Sen Gupta, P. K. ... 180
Seth, P. K. ... 317
Sethi, C. L. ... 69
Sharma, D. C. ... 115
Sharma, L. C. ... 121
Sharma, Rajeswari ... 241
Sharma, S. K. ... 72, 138, 142, 258, 335
Sharma, S. L. ... 42, 333
Shukla, M. ... 178
Siddiqui, M. R. ... 146
Singh, A. ... 254
Singh, G. ... 304
Singh, K. ... 212
Singh, M. ... 23
Singh, R. D. ... 216
Singh, S. ... 72, 142
Sinha, S. ... 63
Sohi, H. S. 42, 317, 333
Solel, Z. ... 46
Srivastava, D. N. ... 77
Srivastava, G. C. .. 249
Srivastava, S. N. S. ... 172
Subba Rao, N. S. ... 183
Suryanarayana, D. ... 188
Swarup, G. ... 69, 88, 133, 212
Thirunavukarasu, V. 202
Tilak, S. T. ... 66
Vasudeva, R. S. ... 296
Verma, B. R. ... 42, 333
Verma, H. N. ... 323
Yadav, A. S. ... 208

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CIBA Pesticides

Dimecron — a systemic insecticide, based on Phosphamidon, is effective against sucking, chewing and mining insects. It is water soluble, odourless, does not taint and most crops are free from residues ten days after treatment.

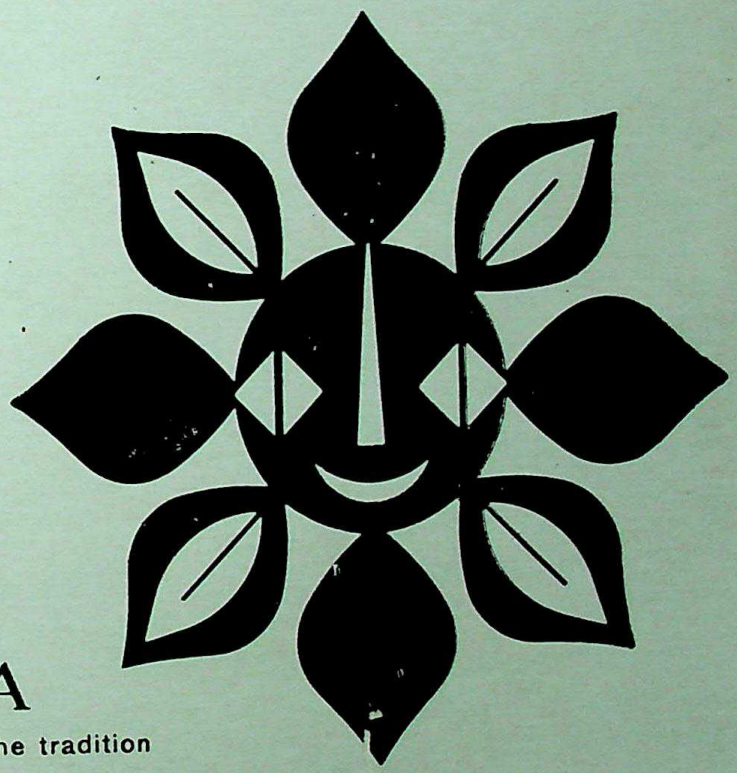
Nuvan — an insecticide with extremely potent fumigant and contact action against household and storage pests as well as against chewing and mining caterpillars on field crops.

Thiocron — an acaricide cum insecticide of low toxicity with specific action against mites.

Cuman — an organic fungicide with excellent rain resistance, suitable for the control of diseases on fruit trees and field crops.

Cosan — a wettable sulphur preparation of colloidal particle size for the control of powdery mildew on fruit crops and against mites.

Copramat — a fungicide, based on a combination of Copper-oxychloride and ziram for the control of diseases on field crops.



C I B A

where research is the tradition

Stocks now available in India.
For detailed information, please write to
CIBA of India Limited, Pesticides Division, P.O.Box 479, Bombay 1

JWT-C.2548A

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(*Contd. from 2nd cover*)

Phytopathological Notes

Chemical sprays for the control of secondary infection of black rot of cauliflower—*Y. P. Rao and S. B. Mathur* ... 332

Control of damping-off of cauliflower—*H. S. Sohi, S. L. Sharma, K. B. Sachdeva and B. R. Verma* 333

Identification of new biotype within Indian race 162 of Puccinia recondita Rob ex Desm—*R. Prasada, S. K. Sharma and M. M. Merchanda* 335

Helminthosporium carbonum Ullstrup, on maize in India—*T. P. Bhowmik and B. L. Chona* 337

Seventeenth Annual Report of the Indian Phytopathological Society, 1963 339

Abstracts of papers read in the Symposium on "Physiology of Host-parasite relationships in plant diseases" during the Seventeenth Annual General Meeting of the Indian Phytopathological Society held at Calcutta, on 29th December, 1964 341

Index to volume XVII .. .. i—viii

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1964

President ...	... S. SINHA
Immediate Past President ...	... T. S. SADASIVAN
Vice-President	... N. PRASAD
Secretary-Treasurer ...	... S. P. RAYCHAUDHURI
Joint Secretary... ...	... D. SURYANARAYANA

COUNCILLORS

Northern Zone ...	... B. L. RENFRO
Mid-Eastern Zone ...	... G. S. VERMA
Eastern Zone ...	... U. N. MOHANTY
Central Zone ...	... B. P. PATIL
Western Zone ...	... K. G. NEMA
Southern Zone ...	... S. B. LAL

EDITORIAL BOARD FOR 1964

R. PRASADA

Editor-in-Chief

S. P. RAYCHAUDHURI
M. J. THIRUMALACHAR
K. S. BHARGAVA
G. RANGASWAMI
KISHAN SINGH
D. N. SRIVASTAVA
M. M. PAYAK

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

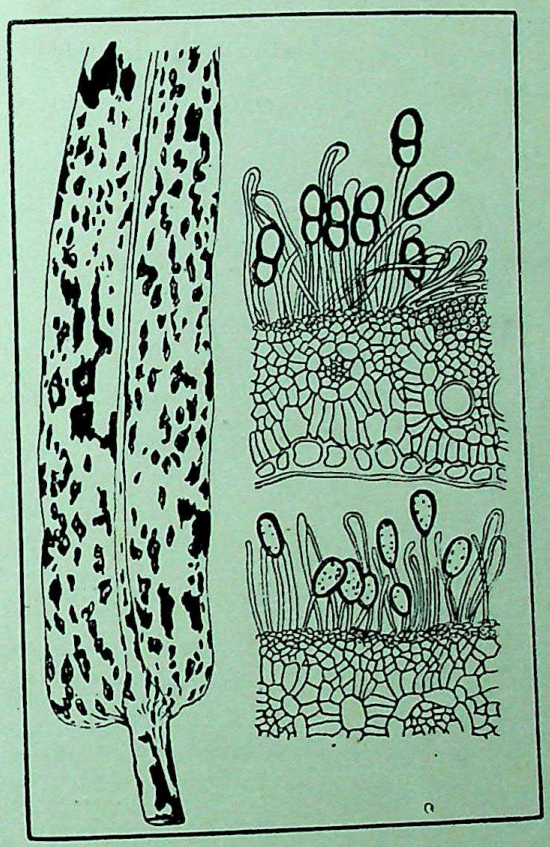
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVIII 1965 NUMBER 1



PUBLISHED FOR
THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Microbiological complex of the Phyllosphere and disease control—*S. Sinha* 1

Carbon requirements of Colletotrichum inamdarii—*S. K. Hasija* 21

On two unrecorded races and a new biotype of Puccinia recondita—*M. M. Payak* 26

A new rust on Schizandra grandiflora—*Hardev Singh and S. Jalan* 29

Seed microflora I. Seed-borne fungi of Ragi (Eleusine coracana Gaertn.) their distribution and control—*J. S. Grewal and Mahendra Pal* 33

Leaf blight and dry stalk rot of strawberry caused by Rhizoctonia bataticola—*V. C. Lele and H. C. Phatak* ... 38

Studies on leaf blight of Inga dulcis—*A. K. Roy* ... 43

Cultivation of some plant viruses in callus tissue cultures—*S. P. Raychaudhuri and M. D. Mishra* 50

The genus Pringsheimia in India—*S. T. Tilak and Ramchandra Rao* 54

Studies on Cape gooseberry mosaic disease—*S. P. Capoor and D. C. Sharma* 58

Rusts connected with Barberry in Central Nepal—*D. P. Misra* 66

Some new records of the Order Mucorales from India—*A. K. Sarbhoy* 71

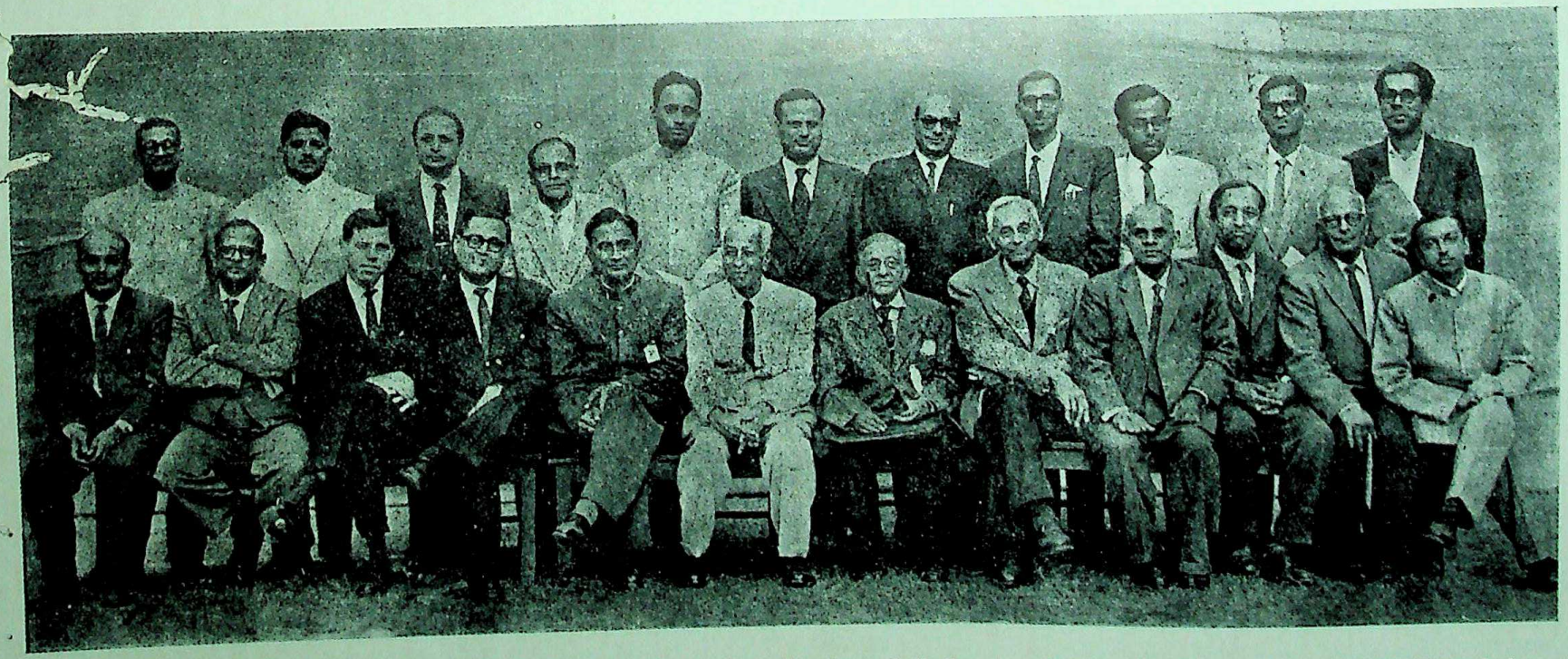
Phytopathological Notes

Inhibition of sporulation by light in Alternaria triticina Prasada and Prabhu—*A. S. Prabhu and R. Prasada* ... 81

(*Contd on. 3rd cover*)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

INDIAN PHYTOPATHOLOGICAL SOCIETY-XVII ANNUAL MEETING 1964, CALCUTTA



Sitting (Lt. to Rt) : G. Rangaswami, S. Y. Padmanabhan, B. L. Renfro, S. P. Raychaudhuri, T. S. Sadasivan, M. J. Narasimhan, S. R. Bose, K. D. Bagchee, S. Sinha, M. J. Thirumalachar, R. Prasada, C. V. Subramanian.

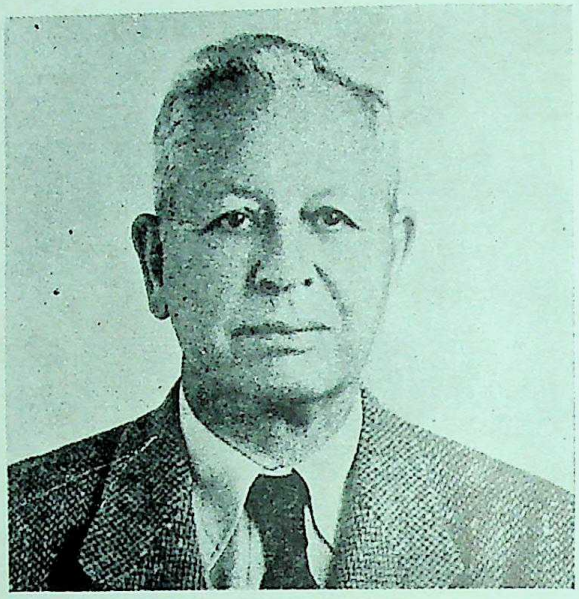
Standing (Lt. to Rt) : G. Lall, A. Appa Rao, S. T. Tilak, C. R. Das, Kishan Singh, S. C. Gupta, H. C. Arya, S. S. Jain, A. Mahadevan, M. R. Shrama, N. K. Chakarabarti.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

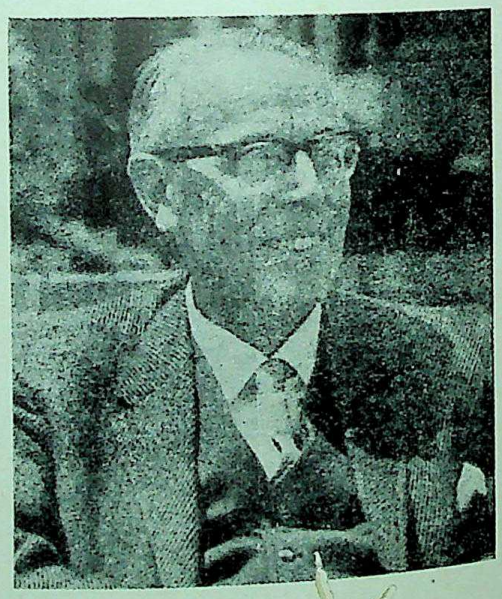
Digitized by Arya Samaj Foundation Chennai and eGangotri

Honorary Fellows of The Indian Phythopathological Society Elected in 1964



E. C. Stakman

S. R. Bose



Kenneth M. Smith

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

E. C. Stakman

Born on May 17, 1885 at Algoma (Wisconsin) U.S.A. Educated at the University of Minnesota, obtained the B.A., M.A. and Ph. D. degrees. Recipient of several honorary degrees from the Universities of Halle-Wittenberg, Germany, Yale, Rhode Island, Minnesota, Wisconsin and Cambridge (England). Joined the Department of Plant Pathology, University of Minnesota in 1909; acted as Chief of the Department from 1940-1953; also served as agent of U. S. D. A. incharge of stem rust epidemiology studies at the Federal Rust Laboratory, St. Paul in cooperation with the University from 1917-1955 ; served as Visiting Professor for short periods at the University of Halle-Wittenberg (Germany). University of California and Indian Agricultural Research Institute, New Delhi (India).

Dr. Stakman was President of several professional societies such as American Association for the Advancement of Science, American Phytopathological Society, etc. He is the recipient of several national and international awards for meritorious service in the field of phytopathology; member of several national and international scientific societies. Holds honorary memberships of more than 15 societies. Has published over 300 articles of scientific or educational nature; is also author of book (with J.G. Harrar) on 'Principles of Plant Pathology' and of chapters in *Advances in Agronomy, Plant Pathology, Problems and Progress* 1908-1958, *Annual Review of Phytopathology* Vol. 2, etc.

S. R. Bose

Born on 15th February 1888, at Nagbal village, Hugli district (Near Chirisurah). Educated at the Presidency College of Calcutta from 1905-1908. Took B.A. degree ('B' course) in 1907 and M.A. degree in 1908, Doctorate in Botany in 1922 of the Calcutta University. Worked at Mycological Laboratory of University of Sorbonne, Institutes Viticultures, Jardin des Plantes (Nat. Hist. Museum), Paris, Botanical Laboratory of the Imperial College of Science, London, Cambridge, Kew gardens, British Museum, Edinburgh, Berlin, Baarn (Holland), Zürich, New York Botanical Garden and Mycological Museum and Syracuse Forestry Institute.

Elected Fellow of Royal Society of Edinburgh (F.R.S.E.) in 1925, of the National Institute of Sciences of India in 1935. Recipient of several prizes in recognition of his mycological researches. Emeritus Professor of Medical Mycology in the School of Tropical Medicine since 1960, and Emeritus Professor of Botany at R.G. Kar Medical College, since 195[illegible]

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Prof. Bose has published 118 original papers on mycological aspects in international journals. He has taken active part in several national and international gatherings. He was elected Vice President of Mycology Section of International Botanical Congress held in Stockholm, 1950; President of Botany Section of the Indian Science Congress in 1938; twice President of the Indian Botanical Society, also of the Indian Phytopathological Society and of the Bengal Botanical Society; Honorary Member and Fellow of the Bengal Botanical Society.

Kenneth M. Smith

Educated at Dulwich College, London University, awarded D.Sc. Manchester, Ph. D. Cambridge, F.R.S. (1938), C.B.E. (1954) first post Senior Lecturer in Agricultural Entomology, Manchester University. Then joined Dr. Salaman at the founding of what was then the Potato Virus Research Station at Cambridge, remained there as Senior Research Officer till 1939 when Dr. Salaman retired. Became Director of the Plant Virus Research Institute (name changed from Potato Virus Research Station). The laboratory was taken over by the Agricultural Research Council in 1948 and renamed Virus Research Unit. Retired from the Directorship in 1959 when Dr. Roy Markham became Director, but continued to work at the Virus Research Unit until 1962 when he was appointed Visiting Professor in the Department of Biophysics, University of Pittsburg, U.S.A. After two years there he moved to the Cell Research Institute and Dept. of Botany, University of Texas, Austin, as Visiting Professor where he is now working.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MICROBIOLOGICAL COMPLEX OF THE PHYLLOSPHERE AND DISEASE CONTROL*

S. SINHA[1]

Botany Department, Agra College, Agra

I am deeply conscious of the honour you have done me, ladies and gentlemen. For this, I thank you most sincerely.

In my talk today I shall concern myself with the microbiological complex of the phyllosphere, particularly in relation to disease in plants. Phyllosphere is to the shoot what rhizosphere is to the root. We have by now a good understanding of the rhizosphere but problems of the phyllosphere are yet to be studied and only beginnings have been made. In the rhizosphere the roots have a complex relationship with the microorganisms in the soil. Similarly, there exists an influence of the shoot, particularly of the leaf, on the microorganisms present on the surface of stems, leaves, flowers, fruits and seeds. The surfaces of the shoot secrete/excrete certain exudates which may directly affect the surface microorganisms, some of which may be pathogenic. The host tissues are also known to exude phytoncides which are inhibitory to the invading fungi and bacteria. Some of the surface microorganisms may cause the production of phytoalexins in the host and bring about changes in the reaction of the host to parasites. A few of the surface microorganisms may be hyperparasites. The microorganisms themselves produce self-inhibitory and self-stimulatory substances bringing about a great influence on their own germination. They also interact and this interaction leads to the suppression or stimulation of one or the other, bringing in antagonistic or associative effects. This complex problem of the phyllosphere has a profound influence on the course of events in the infection of hosts and is intimately related to the formulation of methods of disease control. The study of phyllosphere is still in its infancy but the field of investigation is promising and great phytopathological possibilities are bound to unfold as work proceeds.

In one of the earliest references to phyllosphere, Last (1955) reported occurrence of *Sporobolomyces* on cereal leaves and colonies of these were greatly decreased by *Tilletiopsis*, particularly in July-August on the spring-sown barley. Besides, several other fungi were present on leaves. Simard *et al.* (1958) showed antagonism between some microorganisms isolated from the surface of dead apple leaves and the apple scab fungus, *Venturia inaequalis*. Ruinen (1961) examined the microorganisms of fresh leaves of various plants at Bogor Botanic Garden, Java, and determined the water and nutrient relations of the phyllosphere. Singh and Sinha (1962) reported a number of fungi,

*Presidential Address for 1964 delivered at the meeting of the Indian Phytopathological Society held on 30th December, 1964 at Calcutta.

[1]President of Indian Phytopathological Society for 1964.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

bacteria and actinomycetes from the surface of brinjal, bottle-gourd and lady's finger growing in fields near Agra. These were *Zygorrhynchus*, *Aspergillus niger*, *A. fumigatus*, *A. tamarii*, *A. sydowii*, *Fusarium*, *Monilia*, *Cladosporium*, *Curvularia*, *Streptomyces* spp. and a number of gram-negative bacteria. Some of these were obtained exclusively from either of the three hosts, while others were common to two hosts and still a few others occurred in all the three. Two species of *Fusarium* caused decay of the vege ables. Four surface microorganisms were antagonistic to the two pathogens. El-Helaly *et al.* (1962) studied the fungi associated with onion crop in the field and during storage. Pathogenic species included *Fusarium solani* on seeds and stored bulbs, *F. equiseti* on seedlings, *F. oxysporum* and *F. moniliforme* (*Giberella fujikuroi*) on bulbs, together with *Aspergillus elegans*, *A. niger*, *Rhizopus nigricans*, *R. stolonifer*, *R. nodosus*, *Sclerotium cepivorum*, *S. rolfsii* and *Botrytis septosporia*; *Stemphylium botryosum* (*Pleospora herbarum*) occurred on leaf areas previously attacked by *Peronospora destructor*. Morton and Peterson (1960) reported from field observations that early infections of *Helminthosporium sativum* in leaf blades inhibited later development of *Septoria passerinii* in leaf sheaths. Wibe and Morton (1962) confirmed the field observations that *H. sativum* infections may inhibit *S. passerinii* development, and also indicated that *H. sativum* inhibition by *S. passerinii* is unlikely. In addition, they demonstrated that a cell-free extract of either fungus will inhibit *S. passerinii* development in leaves, suggesting that nonspecific substances may cause some of the observed inhibition although *H. sativum* extract was considerably more inhibitory than that of *S. passerinii* when the entire leaf was inoculated. Rubenchyk *et al.* (1962) studied the phyllosphere of trees in an orchard of Odessa State University. Trees of pear, plum, peach, mazzard cherry and wild vine were found to be less conducive to the development of microorganisms than apricot, Persian walnut, apple and cherry. Among the most characteristic forms of the microorganisms for apricot were mycelial fungi and for walnut, yeast-like cells and actinomycetes. The forms characteristic for each plant persisted for three years. Bier and Rowat (1962 a, b) reported the presence of saprophytes on bark of poplar and willow and these saprophytes were found to be inhibitory to canker caused by *Hypoxylon pruinatum*, and surface sterilization rendered the host cuttings susceptible. In recent studies at Agra College, the phyllosphere microflora on leaves of pearl millet (*Pennisetum typhoideum*) consisted of *Sclerospora graminicola*, *Rhizopus*, *Choanephora cucurbitarum*, *Talaromyces* (2 spp.), *Chaetomium globosum*, *Tolyposporium penicillarieae*, *Trichoderma koningii*, *Aspergillus fumigatus*, *A. nidulans*, *A. sydowii*, *A. flavus*, *A. terreus*, *A. japonicus*, *A. niger*, *Penicillium oxalicum*, *Curvularia verruculosa*, *Helminthosporium australiense*, *Fusarium orthosporioides*, *F. semitectum*, *F. moniliforme* and *Fusarium oxysporum*. Some of these fungi were found to have antagonistic relationship with *Puccinia penniseti* which incites the rust disease of pearl millet. Mridul Goel and Sinha (1961) isolated a number of microorganisms from the surface of vegetable seeds and some of these are well known to produce antibiotic substances. Further work in progress at Agra College on *Eruca sativa*, *Sesamum indicum* and *Linum usitatissimum* has shown that a host of fungi occur on the surface of flowers, fruits and stored seeds. Those isolated from the flowers are *Malustera aeria*, 3

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spp. of *Alternaria*, *Curvularia lunata*, *Nodulisporium didymosporum*, *Rhizopus arrhizus*, *Fusarium moniliforme* var. *subglutinans*, 2 spp. of *Aspergillus*, and *Penicillium steckii*. The fungi present on the fruits are *Malustela aeria*, 3 spp. of *Alternaria*, *Curvularia lunata*, *Cladosporium sphaerospermum*. 2 spp. of *Aspergillus* and *Penicillium lilacinum*. The surface microflora on the stored seeds consisted of 11 species of *Aspergillus*, *Spicaria divaricata*, *Trichoderma koningii*, *Chaetomium globosum*, *Syncephalastrum racemosum*, *Rhizopus arrhizus*, *Mucor hiemalis*, *Nodulisporium*, *Cladosporium sphaerospermum*, 2 spp. of *Helminthosporium*, 5 spp. of *Penicillium*, *Stachybotrys atra*, *Malustela aeria*, 2 spp. of *Alternaria*, 2 spp. of *Curvularia*, 5 spp. of *Fusarium* and some bacteria and actinomycetes.

In another recent investigation at the same college on the die-back and *Alternaria*-blight of chillies, it has been shown that in addition to 7 actinomycetes and 3 bacteria several fungi are present in considerable concentration on the surface of the shoots. These are *Aspergillus*, *Alternaria*, *Fusarium*, *Heterosporium*, *Helminthosporium*, *Rhizopus*, *Cladosporium*, *Trichorderma*, *Curvularia*, *Penicillium* and *Mucor*. It was observed that the population of these microorganisms shows a close relationship on the plant surface and in the atmosphere. But the presence of *Alternaria* was controlled by a bacterium which is antagonistic towards the former. From March to June, during the young stage of the crop, this bacterium was quite frequent, both on the plant surface and in the atmosphere. The *Alternaria* sp. was proportionately less frequent. The disease intensity also was low. From July onwards, there was a gradual decline in the frequency of the bacterium, and accordingly the *Alternaria* and along with it the disease intensity, increased. Similarly, during winter, the presence of *Colletotrichum capsici* on chillies and the disease caused by it is controlled by certain actinomycetes. It thus appears that some members of the phyllosphere have a protective role to play.

The surface microflora is very much under the influence of the host on which it occurs. There is definite exosmosis (Arens, 1929; Engel, 1939; Hafiz, 1952) of materials from the external surface of plants which is parallel to root exudates in the soil. The moisture film on plant surfaces inevitably carries both organic and inorganic substances (Brown 1922, 1936) in solution, released from the underlying plant tissues and these have been shown to influence germination. *Cicer arietinum* for example, secretes malic acid from glandular hairs (Hafiz, 1952) which inhibits spore germination of *Ascochyta rabiei*. Different wheat varieties were shown recently (Van Velsen, 1957) to depress germination of *Helminthosporium sorokinianum* on their leaves. Parallel depression resulted when the spores were germinated in leaf secretions from these varieties. There are insufficient data as yet to suggest how wheat leaf secretions act, and whether they are specific in action, but it is reasonable to expect interactions similar to those suggested for the influence of root exudates. Kovacs (1955) found that low incidence of local lesions of sugarbeet leaf-spot on a resistant host variety was correlated with the presence of diffusible inhibitors from healthy leaves. For *Plasmopara viticola*, Arens (1929) showed that epidermal excretions played an important part in establishing infection,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and their positive chemotaxis on epidermal strips occurred only when the guard cells were alive. The activity coming from guard cells was attributed to surface active materials accumulating in the interphase between stomatal gas and infection drop. Evidence has been presented by Martin *et al.* (1957) that inhibitory substances (waxes) obtained from the surface of apple leaves resistant to powdery mildew can confer resistance when redeposited on otherwise susceptible leaves. Similarly, *Vicia faba* leaves were protected against *Botrytis fabae*. The main compound separated by chromatography was a phenol resembling a fungitoxic substance which has appeared in water washings of many trees and in the leaf excretion of *Vicia faba* (Topps and Wain, 1957). Petrushova and Meshchaninova (1961), working at the Nikita State Botanical Gardens, Yalta, showed that sap from a number of species of *Clematis* was fungicidal to *Ascochyta pisi*, *Aspergillus niger*, *Helminthosporium cynodontis*, *Monilinia cinerea* (*Sclerotinia laxa*), *Phoma punicae*, *Phomopsis cinerescens*, *Pythium intermedium*, *Rhizoctonia* (*Corti ium*) *solani*, *Saprolegnia* sp. and *Trichothecium roseum*. Loeschke and Butin (1961) at the Inst für Forstpflanzenkrankheiten, Hann, Hunden, found bark extracts of different poplar varieties to be fungistatic to *Dothichiza* (*Cryptodiaporthe*) *populea*. Timonin (1962) reported that the contents of scent glands of *Banasa dimidiata*, *Podisus modestus* and *Brachynema quadripustulata* produced zones of inhibition on potato dextrose agar plates of *Fusarium* sp., *Pythium* sp., *Phytophthora* sp. and *Rhizoctonia* sp. Fawcett (1963) demonstrated the presence of antifungal compounds in plants. Some antifungal compounds are only slightly inhibitory *in vitro* to a pathogen of a host, but may be severely inhibitory to other fungi which are unable to attack that host.

Several hosts are known to contain preformed chemical compounds which are inhibitory to pathogens and are termed phytoncides. Some of these may be volatile and influence the microorganisms present on the surface of host plants. Nikitina (1962) conducted a four-year study at the Leningard Institute of Soviet Trade and found accumulation of phytoncides in leaves and fruits of apple and pear varieties with different maturation times and their action against bacteria, *Penicillium glaucum*, *Trichothecium roseum*, *Mucor mucedo*, *Monilia fructigena*, etc. The phytoncides were less active in the leaves of apple seedlings than in those of fruit bearing trees. Apple leaves of all the varieties were more phytoncidal during mid-July to mid-August, and the fruit at the time of technical maturity. The most active volatile fraction occurred in the leaves of pear seedlings and fruit bearing trees, the phytoncides of which killed bacteria and fungi throughout the growing period. Kuć *et al.* (1959) separated by chromatography two compounds from extracts of Idako-grown Netted Gem potato peel and pulp tissue. These substances were fungistatic to *Helminthosporium carbonum* and their physical and chemical properties resembled those of chlorogenic and caffeic acids which are presumed to be closely associated with natural immunity from *H. carbonum* in potatoes. The bacteristatic effects of phytoncides in volatile and non-volatile fractions of extracts of *Allium sativum* and *A. cepa* were studied at Tashkent by Abdullaeva (1962). The volatile fractions produced a sterile zone in cultures of *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum*,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Xanthomonas malvacearum and *Rhizoctonia* sp. Tokin (1960) called the phytoncides as destroyers of microbes, and reviewed the work of other workers in Russia. Among the results cited are those of Mme. F. V. Khetagurova and Mme. V. G. Gramenitskaya with volatile phytoncides from different parts of bird cherry (*Prunus padus*), oak, cherry, laurel, pine, larch, etc., which were bactericidal in various degrees to *Pseudomonas citriputeale* on citrus and other plants. Good control of rose rust is reported by I. V. Michurin with sap from *Mulgedium*, the phytoncide of onion also being used. That of onion and horse-radish gave effective control of barley smut and of seed-borne diseases of cotton. Mme. K. I. Beltyukova in researches over 10 years secured inhibition of phytopathogenic bacteria with plant products such as that from waterlily (*Nuphar* sp.) even at very low concentrations. N. Krasil' Nikov has shown that some plant diseases could be controlled and infection averted by adding phytoncides from lower plants to the plants' own phytoncide. Mme. Z A. Borzova showed that spores of *Phytophthora infestans* were killed by volatile phytoncides of onion bulbs and garlic, fir needles and leaves of cherry and citrus. Dilutions of plant tissue sap were also highly effective against *Phytophthora infestans*. A water infusion of dry surface scales of onion and garlic immediately killed spores of *P. infestans*. Mme. V. G. Gramenitskaya obtained excellent results with phytoncides of mustard, horse-radish and *Eucalyptus* as a treatment for cotton seed against *Xanthomonas malvacearum*. O. Savchuk found that various fruits could be preserved longer in an atmosphere of volatile phytoncides of horse-radish. Other experiments showed that carrots could be preserved by spraying with onion sap or with a water infusion. A phytoncidal preparation from wheat grains preserved lemons. N. S. Burev obtained excellent results in large scale experiments with newly picked apples and wind falls by treatment with water infusions of the outer dry scales of onion.

Phyllosphere microflora is likely to produce phytoalexins as a result of the infection of the host by microorganisms, thereby rendering the host resistant to parasites. Phytoalexins are fungistatic or antibiotic principles yet to be understood. Bernard (1909) appears to be the first to put forth this concept in which local saprophytic infection may provide protection against subsequent infections of the host. He found that if orchid seedlings were infected by a mycorrhizal fungus, they became resistant to attack by other pathogens. Further evidence on the formation of phytoalexins was provided by Müller and his co-workers. They observed that potato that had been invaded by an avirulent strain of *Phytophthora* became resistant to the virulent strain of the parasite and the changes undergone by the tissue under the influence of a virulent strain rendered it inhospitable to some other potato parasites. Their results indicate that in the course of interaction between the host and the avirulent pathogen, a factor is activated in the former which exerts an antibiotic effect on the latter, and the greater the speed with which the factor appears in the infected host tissue, the earlier is the pathogen checked. This hypothetical nonspecific principle has been termed as 'phytoalexin' (PA), a designation which refers to its biological action only and does not include any suggestion as to its chemical nature. The idea of the formation of antipathogenic principles by host plants is

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

supported by the Gäumann school (1951, 1956) in Switzerland, Kuć team (1956) in U.S.A., Uritani and Akazawa (1951) and Uehara (1959-1962) in Japan and Cruickshank and Perrin (1961 and 1963) in Australia. Kuć *et al.* (1956) observed that when slices of potato tubers were inoculated with *Helminthosporium* species non-virulent to this host, antibiotic factors developed which checked not only the pathogen but other fungi as well. They found an increased polyphenolic (chlorogenic acid and caffeic acid) content in the inoculated tissue. In an experiment with young pods of bean (*Phaseolus vulgaris*) inoculated with spore suspension of *Phytophthora infestans* or *Sclerotinia fructicola*, Müller (1956) noticed that the inoculated areas react with necrotic flecks caused by the pathogen and at the same time the pathogen ceases to grow. It was shown that in the course of interaction, considerable amount of a toxic principle (PA) diffuses into the inoculation droplets, which is absent in the noninoculated tissue at concentrations inhibitory to the pathogen. Phytoalexin activity was not influenced by chemical factors in the host tissues which may serve as nutrients to the pathogen. The mechanism underlying phytoalexin formation appears to be a general phenomenon, the same mechanism having been found in nine other host-pathogen combinations (Müller, 1958). Jerome and Müller (1958b) showed that the resistance of pods of *Phaseolus vulgaris* to *Sclerotinia fructicola* and *Botrytis cinerea* is affected by temperature of the environment before inoculation. At the critical temperature of 44°C for two hours, the loss of resistance is reversible. Recovery occurs in three days when the material is stored at 20°C. The output of phytoalexin is also affected by preinoculation conditions at various temperatures, and there is always a close relation between the clinical behaviour of pods and the production of the antibiotic principle. Uehara (1958) worked on properties of phytoalexin produced as a result of the interaction between pea (*Pisum sativum*) and *Ascochyta pisi*. The phytoalexin activity was lost after irradiation by UV-light (1850-5780Å) at 45 cm. distance for 45-60 minutes. Uehara (1962) estimated production of the phytoalexin by pea pods by means of ultra violet absorption spectrum. The UV absorption spectrum of the extract from pods and leaves of naturally infected pea plants did not differ from that of an extract from the phytoalexin solution. Cruickshank and Perrin (1961) gave an account of the isolation of pisatin from detatched pea pods inoculated with *Monilinia* (*Sclerotinia*) *fructicola*. In further work on pisatin production they showed that the concentration of pisatin formed after inoculation was uniformly proportionate to pod maturity. Storage of pods aerobiologically at 4°C ensured the best relation of the ability to form inhibitory levels of pisatin, the total concentration and rate of formation of which were affected by inoculation concentration and post-inoculation temperature. Thirteen facultative fungi and six obligate species were tested for pisatin production and all of them reacted to form the phytoalexin. Pisatin concentrations of >ED50 value were induced by non-pathogens and concentrations <ED50 by pathogens of peas and the reaction of the endocarp of pea pods depended upon sensitivity of the pathogens to the concentration of pisatin formed in the early days of inoculation. They concluded that a host gives a resistant reaction when a given infection stimulates sufficient phytoalexin to inhibit the pathogen and susceptibility may be the result of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the tolerance of phytoalexin by the pathogen or its inability to stimulate phytoalexin production.

The phyllosphere microflora is not only under the influence of the host, but is also subject to its own influences. An important aspect is the production of self-inhibitory and self-stimulatory products by the microorganisms present in the phyllosphere. However, so far, this phenomenon has been worked out mostly in rusts.

Dickson (1953) was the first to report that germinating rust spores produce a volatile substance, which under certain conditions inhibits germ tube elongation. Allen (1955) studied the phenomenon of self-inhibition in greater detail in *Puccinia graminis* var. *tritici*. He collected evidence in favour of the volatile nature of the inhibitor, which was found to be heat-stable, adsorbed or otherwise removed from solution by glass surfaces, and to be more effective at high than at low pH. Similar observations have also been made by Forsyth (1955), indicating that uredospores of *P. graminis* var. *tritici* produce a volatile substance which was identified as trimethyl ethylene by comparing the absorption spectrum of the natural inhibitor with that of trimethyl ethylene. The same author discovered four ways of overcoming self-inhibition, using silver nitrate, ammonia, acetone solution and increased water volume in relation to uredospores. Yarwood (1954) reported that attachment of rusted bean leaves to the leaves inoculated with bean powdery mildew, bean anthracnose, tobacoo mosaic virus and tobacoo necrosis virus did not reduce infection. However, rusted bean leaves attached to leaves freshly inoculated with bean or snapdragon rust, reduced the infection. He opined that the active materials of the aqueous diffusates of uredospore germination are gaseous in nature and toxic to the germinating spores of certain rusts, but not to the other plant pathogens. Later, he (1956) demonstrated a relation between increasing dosages of uredospores and their percentage germination, while investigating simultaneous self-stimulation and self-inhibition during germination of uredospores in *Uromyces phaseoli*. Le Roux and Dickson (1957) showed self-inhibition of germination in uredospores, as well as in aeciospores, of *Puccinia sorghi*. They found that the effect of the environment on the synthesis and action of the inhibitor is closely associated with the influence of environment on spore germination. The inhibitor, according to them, was active in varying degrees, depending upon the specific lot of spores, storage conditions and the period stored for.

Wilson (1958) reported a dosage response with aqueous diffusates from uredospores of bean rust, *Uromyces phaseoli* var. *typica*, against infection with the same. He also demonstrated the presence of aspartic and glutamic acids in the aqueous diffusates of uredospores of bean rust, using chromatographic analysis technique. Quantitative chromatography revealed these acids in sufficient quantities which may account for self-inhibition of uredospore germination. Farkas and Ledingham (1958) studied the relation of self-inhibition to oxidative metabolism of stem rust uredospores. They reported that erratic germination, often leading to nonreproducible experimental results, is due to a highly sensitive interplay between stimulatory and inhibitory substances governing

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

germination. Their results indicate that initial lipid type of metabolism in the rust uredospores changes to a carbohydrate type of metabolism during germination, and this apparently makes it possible for the rust to take advantage of the stimulated carbohydrate metabolism and breakdown in the host. Hoyer (1962) showed that the water used for washing uredospores of *Puccinia recondita* contains a germination inhibitor, which is formed in mature resting spores and diffuses into the medium, even when the oxygen supply has been cut off and the spores are dead. A self-inhibitor was also detected by him in *Puccinia dispersa*, *P. sorghi*, *P. antirrhini* and *Uromyces viciae-fabae*. He reported that the agent is not specific in action and the spores of different species are inhibited mutually, although the intensity of inhibition varies. He noted a periodicity in the production of the inhibitory substance which was highest from December to March and lowest from June to September. According to him this fluctuation, though correlated with weather conditions in general, could not be directly attributed to any other component factors such as light, temperature or air pressure. Recently, Bhowmik and Prasada (1963) found self-inhibition of uredospore germination in the maize rust, *Puccinia sorghi*. Their technique involved attachment of rusted maize leaves, or leaves of wheat infected with *P. graminis* var. *tritici*, in close contact with freshly inoculated maize leaves, when there resulted a zone of rust inhibition, corresponding closely in size and shape to the attached leaves. Maize rust did not inhibit the rust infection of wheat leaves. On the other hand, the inhibitory substance given out by maize rust pustules inhibited the germinative and infective capacity of its own spores. The inhibitor, as they report, was not gaseous in nature and glutamic and aspartic acids, earlier reported by Wilson (1958) in the case of *Uromyces phaseoli*, were not able to inhibit uredospore germination of maize rust even at 500 ppm.

Self-inhibition in uredospore germination has also been demonstrated in *Puccinia penniseti* at Agra College. The uredospore quantity in relation to water volume has profound influence on the rate of self-inhibition. The substance responsible for the inhibition of germination appears to be volatile and heat stable in nature. The absorption spectrum of the inhibitor in 0.5 per cent acetone solution is noticed in ultraviolet range (210-230μ wave length). The inhibitor resembles trimethyl ethylene in its properties. Self-stimulation of uredospore germination has also been noted when the centrifuged inhibitor solution, obtained after germination test, was used in hanging drop cultures.

Apart from the self-inhibitory and self-stimulatory problems, the phyllosphere microorganisms have also an interacting influence, as is found among the microorganisms of the rhizosphere. In this interaction, particularly with the parasitic forms, lies the importance of studies on phyllosphere. Sometimes a fungus or a bacterium present on the shoot surface may be parasitic on the pathogen and shows relationships of hyperparasitism. In other cases, antagonistic or associative effects may exist between the microorganisms of the phyllosphere microflora.

Cases of hyperparasitism are well known in phytopathological literature. *Ciccinobolus cesatii* develops as thin mycelial threads inside

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the hyphae and conidiophores of *Erysiphe graminis*. *C. ewonyi-japonici* on *Oidium ewonyi-japonici* and *C. asteris* on *Oidium astericolum* act in a similar way. *Darluca filum* is reported in the uredo and teleuto-sori of several rusts. Several species of *Tuberculina* are reported to attack members of the Uredinales. Mention may also be made of *Cladosporium aecidiicola* growing in aecia of *Puccinia conspicua* (Keener, 1954) and of *Verticillium hemiteiae* (Bourriquet, 1946). Some bacteria are also known to parasitize rust sori. *Erwinia urediniolytica* attacks pedicels of rust spores (Borders, 1938). *Xanthomonas uredovorus* parasitizes *Puccinia graminis* (Pon *et al.*, 1954).

A more prevalent phenomenon of the phyllosphere is antagonism between the invading pathogen and the microorganisms present on the surface of stem, leaves, flowers, fruits and seeds, a brief reference to which has been made earlier. This aspect of the phyllosphere is important as these studies have opened up possibilities of biological control of plant diseases. The mixing of antagonistic microorganisms with the inoculum of a pathogen can reduce the infection. Bamberg (1931) showed that a bacterium isolated from corn when mixed with smut spores of *Ustilago zeae* reduced the infection rate of corn and inhibited the germination of chlamydospores. Sometimes saprophytes colonize wounds on plant surfaces and show antagonistic reaction with the invading wound parasites. Thus, *Stereum purpureum* establishes itself easily on freshly-cut tissues but does so with difficulty on old lesions that are already colonised by saprophytic microorganisms. Bier (1962) found that two common saprophytes present in the irregularities of the bark of 1-3 year old poplar and willow were inhibitory to *Hypoxylon pruinatum* which is the cause of canker disease of these trees. The pathogen did not develop on cuttings inoculated with the saprophytes, whereas surface sterilization of the cuttings rendered them susceptible to canker. On such evidence, Bier (1963) has indicated the possibility of biolgical control of some tree diseases. Lettuce infected in the field with *Botrytis cinerea* showed greater survival in the hollows than where the ground was flat (Wood, 1951) It was found that in the hollows, dried leaves at the base of plants were sometimes covered with water which had run from the surrounding soil. This permitted the growth of saprophytes, some of which were found to be antagonistic to *Botrytis*. In a similar way, Newhook (1951, 1957) isolated organisms antagonistic to *B. cinerea* from lettuce and tomato. Cultures of *Bacillus*, *Pseudomonas* and *Chromobacterium* from lettuce leaves were strongly antagonistic to *B. cinerea*. Attempts to establish an antagonistic saprophyte on dead tissues of the host has given some results. *B. cinerea* attacks the fruit only after becoming established on dead petals. In green-house experiments, Newhook (1957) applied spores of antagonistic saprophytes of *B. cinerea*, especially *Cladosporium herbarum* and one species of *Penicillium* to tomato petals directly after the fruit was set. This was completely successful when recently dried petals were treated but only 30 per cent successful when applied to petals that had dried for several days.

Natural colonization occurs of organs that have been dead for some time. If these saprophytes are active antagonists to the pathogen, a

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

natural protection results. Sometimes treatments have an indirect effect on colonization of host organs by saprophytes. Newhook reports that if growth substances are applied to avoid fall of tomato fruit, natural colonization with *Cladosporium herbarum* and a *Penicillium* is favoured. Then the infection of *Botrytis* is reduced from about 50 per cent on untreated plants to about 20 per cent on treated plants. At the Moscow Branch of All-Union Institute of Microbiology, 13 of 880 isolates of epiphytic microorganisms on strawberry were antagonistic to *B. cinerea* (Vozyakovskaya and Shirokov, 1961). In laboratory tests, one spray of strawberry fruits with a suspension of spores of *B. cinerea* and antagonistic bacteria reduced infection from 60 to 10 per cent. Bhatt and Vaughan (1963) isolated *Cladosporium*, *Botrytis*, *Dendrophoma*, *Pullularia*, *Fusarium*, *Phoma*, *Penicillium*, *Gnomonia* and *Pestalotia* from strawberry fruits. When plants at the late bloom stage were treated with spore suspensions of *Cladosporium herbarum*, *Aspergillus pullulans* or *Penicillium* sp., the control of *B. cinerea* was 42, 31 and 4 per cent, respectively. With simultaneous inoculation of detatched fruits with *Dendrophoma* and *B. cinerea*, there was 2.4 per cent greymould on the green and 35.3 per cent on the ripe fruit, compared with 80.4 per cent and 89.6 per cent, respectively, in the controls. Spraying in the field with *Cladosporium herbarum* spore suspensions or fungicides did not decrease rot, but the yield of marketable berries was highest from *Cladosporium*-treated plots. It is concluded that primary infection of senescent strawberry flowers by *B. cinerea* is prevented by (a) high pH unfavourable for the production of pectolytic enzymes, (b) presence of toxins or antibiotics, and (c) utilization of infection sites by saprophytes. Simard *et al.* (1958) reported antagonism between the apple scab pathogen, *Venturia inaequalis* and three *Penicillium* isolates found on dead leaves of apple. Singh and Sinha (1962) at Agra reported a number of microorganisms from surfaces of fruits of brinjal, bottlegourd and lady's finger. They found a bacterium antagonistic to a species of *Fusarium* causing soft rot of bottle-gourd, another bacterium and a species of *Streptomyces* antagonistic to a non-sporulating fungus pathogenic to lady's finger and yet another species of *Streptomyces* antagonistic to a *Fusarium* producing soft rot of brinjal. In recent work at Agra College, microorganisms on the surface of leaves of pearl millet (*Pennisetum typhoideum*) have been found to be antagonistic to the rust fungus, *Puccinia penniseti*. Uredospore germination is reduced to 50 per cent when all the surface fungi are taken together. The study of individual effect of the surface fungi in 'fresh' and '48-hours old' suspensions reveals that *Chaetomium globosum*, *Trichoderma koningii*, *Aspergillus japonicus* and *Fusarium oxysporum* depress the germination of uredospores by over 80 per cent or so. All these four organisms also control the development of rust pustules on host seedlings in varying degrees, but the most successful effect is that of *Chaetomium globosum*. Cases of antagonism at the time of sowing seeds have also come to light. The normal saprophytic flora on the surface of seeds limits the attack of certain pathogens. Simmonds (1947) reported that the percentage of corn seedlings attacked by *Helminthosporium sativum* was decreased by incubating the seeds for 24 hours in a humid room before inoculation. Probably the moisture favoured the growth of antagonistic saprophytes on the integuments of the seed. Ledingham *et al.* (1949) and Sallans

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

et. al. (1949) demonstrated that when chemical treatment removed the surface flora from seeds, there was an increase in attack by *H. sativum.* Formaldehyde or lactic acid acted in this way. Thus, the seedlings from treated seeds were more frequently diseased than were those from untreated seeds, especially in sterile soil. In pure culture, many organisms which are antagonistic to pathogens have been isolated from the surface of the seed integuments. Attempts have been made to utilize these or other organisms in fighting pathogens that attack seeds. Novogrudski (1937) recommended bacterial inoculation of seeds to combat *Fusarium lini* and *Colletotrichum linicola* on flax. The attack of *Fusarium gramineum* was reduced by treating corn seed with cultures of *Pseudomonas* or *Achromobactor* (Beresova and Naumova, 1939; Krasil' Nikov and Raznitzyna, 1946). By using *Chaetomium cochlioides*, Tveit and Wood (1955) obtained as good a control of the blight of barley seedlings, caused by *Fusarium nivale*, as when organo-mercurials were used. Apparently, the antagonist was able to establish itself and to persist for some time in the soil. Production of antibiotic substances in the integuments of seeds was studied by Wright (1956c). He demonstrated that *Penicillium frequentans* could produce frequentin and *Streptomyces venezuelae*, an unidentified bacterial substance that seems to differ from chloromycetin. Tyner and Mckinnon (1964) observed that various seed-borne fungi of barley, including the pathogen *Cochliobolus sativus* (*Helminthosporium sativum*), were not eliminated from barley seed treated by methods known to eliminate *Ustilago nuda*. The presence of *C. sativus* was readily masked by other fungi present, but holding moist seed under anaerobic conditions for 48-64 hours before plating showed high incidence of the pathogen. When spores of *Gliocladium roseum* or *Trichoderma viride* were examined with spores of *C. sativus* and added to barley seed, pathogenicity of *C. sativus* was reduced. Leben (1964) obtained 230 bacterial isolates from leaves of cucumber seedlings grown from seed in a humid isolator. He tested these in the green house as protectants for the control of cucumber anthracnose and powdery mildew, and found one of them, *i.e.*, A 180, to consistently decrease anthracnose. However, not one of these isolates affected the powdery mildew.

The occurrence of these cases of antagonism in nature points to a future where the antagonistic surface microflora might be employed to control various pests and diseases. Tubeuf (1930) reported success in controlling blister rust of pines in Germany. He placed spores of the parasite *Tuberculina maxima* on aecial fruiting structures of the rust fungus, at strategic spots of infested wood. Tubeuf claimed that the disease was greatly reduced by preventing spore formation and spread of the pathogen.

So far, only a few antifungal substances have been isolated, apparently because the search for them has not been as intensive as for antibacterial substances of potential medicinal value. In 1932, Weindling noted the antagonism of *Gliocladium fimbriatum* to a number of fungi. In 1934, he studied a lethal principle produced by this organism and found that it was responsible for the parasitic action of *G. fimbriatum* on *Rhizoctonia solani* and other fungi. Two years later, Weindling and Emerson (1936) isolated in crystalline form the substance known as

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

gliotoxin, thus becoming the first to isolate an antibiotic in pure state. Later studies showed that gliotoxin reduced infection of covered smut of barley, bunt of wheat and *Helminthosporium* leaf-spot of oats. The next report of the control of plant disease by antibiotic was by Brown and Boyle (1944) who observed that a crude culture filtrate of *Penicillium notatum* was effctive against crown gall, caused by *Agrobacterium tumefaciens*. In 1946, Brown and Heep reported the eradication of *Xanthomonas pruni* from cankers on plum twigs. It was not until 1952, however, that an antibiotic was used under field conditions for the control of plant disease. Zaumeyer *et al.* (1953) showed that beans were protected from interaction by the halo blight organism (*Pseudomonas phaseolicola*) with a streptomycin spray and Ark (1953) reported good control of pear blight (*Erwinia amylovora*) with a streptomycin dust.

The promise of these early reports encouraged further study so that there exists today a voluminous literature describing laboratory, greenhouse and field studies of the effectiveness of antibiotics in plant disease control. An understanding of the selective toxicity, absorption and translocation, and mode of action of antibiotics is necessary if these substances are to be effectively employed for the control of plant diseases. The antibiotic must be toxic to plant pathogenic bacteria, fungi and/or viruses and must demonstrate little or no phytotoxicity to the host when applied at concentrations that inhibit or destroy the pathogen. The phytotoxicity of antibiotics has been assessed by a number of procedures, and the detailed discussions of the various influences of antibiotics on plants have been prepared by Brian (1957). Most antibiotics examined have been found to be phytotoxic to some extent. The degree and type of primary injury observed has varied with their nature, concentration and method of application. However, microorganisms appear to be more sensitive to antibiotics than higher plants and the more toxic antibiotics are capable of controlling disease organism at concentrations that do not visibly affect plant growth.

Various methods of application of antibiotics to plants have been employed, *viz.*, by sprays, dusts, dips, injections, soil and sand applications, paints and pastes. The more practical method of application for the control of plant disease is by foliar sprays. Consequently, the absorption and translocation of antibiotics by leaves are receiving increased attention (Pramer, 1959). The mode of action of an antibiotic is not necessarily a fixed character of the compound. The plant itself may exert a controlling influence. This point was emphasized by Hilborn (1953) who demonstrated different effects produced by the same antibiotic on the same pathogen in different hosts. The resistance of streptomycin treated potato plants to infection by *Phytophthora infestans* appears due to the ability of the antibiotic to stimulate the plant phenoloxidase system to produce quinones at tissue concentrations that inhibit the pathogen (Vörös *et al.*, 1957).

There is evidence that some antibiotics are degraded by plants (Crowdy *et al.*, 1956; Sanwal, 1956; Prescott *et al.*, 1956), and in one case streptomycin amine and streptomycin oxine were converted in plants to compounds of an unknown nature that demonstrated increased

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

activity (Gray, 1958). Although the literature provides no example of an antibiotic which controls disease by toxin inactivation, it is a possibility that must be considered. Likewise, the fact that an antibiotic may have 2 or more modes of action cannot be ignored.

The high cost of antibiotics, while not limiting their use for the saving of human lives, would exclude their use in the control of plant diseases, especially when many synthetic fungicides are available. Nevertheless, the stimulus to further antibiotic research in plant pathology, is the occurrence of certain types of diseases which cannot be controlled by the usual organic and inorganic fungicides. Outstanding examples of such antibiotics are the polyenic antibiotics. Their importance in the protection of man, animal and plant is growing. They are used for the fight against different pathogens, for the preservation of fruits (*e.g.*, for the protection of peaches against *Sclerotinia fructicola*) and vegetables, and in the fermenting industries to prevent unwanted side-fermentations.

The most important antibiotics of this group are candicidin, helixin (endomycin), nystatin (mycostatin), rimocidin, trichomycin, polyenes contained in filtrates of actinomycetes. It seems that the relation between *dosis toxica* to *dosis curativa* is very good.

Helminthosporium of poppy is often the limiting factor of poppy culture in central Germany. At times, seeds are infected up to 95 per cent. The pathogen is inside and outside the seed, so the treatments with mercury containing compounds do not always prove satisfactory. In plate diffusion tests, Klinkowsky *et al.* (1959) showed that seeds take up the antibiotic. Hence the seeds were dipped for two hours in sterile filtered culture media, dried and drilled. Field trials lasting 6 years proved the success of the treatment. The known fungicides do not have this effect.

In other tests, Klinkowsky *et al.* (1959) used the active principles as spraying agents. In trials lasting several years, they treated apples against mildew (*Podosphaera leucotricha*). Other tests were concerned with mildew of roses and the black rot (*Actinonema rosa*) of roses, and since 1958, against the fruit rot of tomatoes (*Alternaria solani* and *Phytophthora infestans*).

Apple mildew has been of growing importance in central Europe. Since the pathogen winters under the bud scales, the treatment with wettable sulphur has no effect; it is effective only against secondary infection. For the treatment of primary infection, there is only trimming of the infected twigs. Klinkowsky *et al.* studied the effect of antibiotic treatment against primary and secondary infections. Spraying was performed at fortnightly intervals; before, during and after the blossom. They succeeded in getting almost full control of mildew infestation.

The activity of antibiotics containing culture filtrates against loose smut of barley and wheat (*Ustilago nuda* and *U. tritici*) were tested

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

with the spore germinating test. The filtrates in dilutions of 1 : 25 to 1 : 50 stopped spore germination completely. They have strains which, besides good activity against spores, gave no decrease of seed germination even after treatments of 8 hours and longer. For the control of loose smut of wheat and barley in the field with these strains, there are still some problems.

The use of polyenes will make it possible to fight pathogens which were not satisfactorily fought with the known fungicides, or not fought at all.

Furthermore, a study of the antibiotics that are known to be fungitoxic or bacteristatic might provide models for designing molecules that will operate as systemic therapeutants, and thereby open up an entirely new avenue of investigation.

Although progress to date is limited, it is obvious that a new era is on the horizon of plant chemotherapy.

References

ABDULLAEAVA, Mme A. (1962) Effect of phytoncides of onion and garlic on some groups of microorganisms. *Uzbek. biol. zh.*, **6** : 40-45.

ALLEN, P. J. (1955) The role of a self-inhibitor in the germination of rust uredospores. *Phytopathology*, **45** : 259-266.

ARENS, K. (1929) Physiologische Untersuchungen an *Plasmopara viticola* unter besonderer Berücksichtigung der Infektionsbedingungen. *Jahrb. wiss. Bot.*, **70** : 93-157.

ARK, P. A. (1953) *Plant Dis. Reptr.*, **37** : 404-406.

BAMBERG, R. H. (1931) Bacterial antibiotic to *Ustilago zeae*. *Phytopathology*, **21** : 881-890.

BERNARD, N. (1909) *Ann. Sci. nat. Bot.*, **9** : 1-196.

BHATT, DIBYA D. AND E. K. VAUGHAN, (1963) Interrelationships among fungi associated with strawberries in Oregon. *Phytopathology*, **53** : 217-220.

BHOWMIK, T. AND R. PRASADA (1963) Self-inhibition of uredospore germination in maize rust, *Puccinia sorghi* Schw. *Indian Phytopath.*, **16** : 195-200.

BIER, J. E. (1962) Actioione and natural bark extracts in the control of *Hypoxylon* canker of poplar. *For. Chron.*, **38** : 363-365.

——— (1963) The saprophytes and the possibility of biological control of some tree diseases. *For. Chron.*, **39** : 82-84.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

——— AND MARIAN H. ROWAT (1962) The relation of bark moisture to the development of canker diseases, caused by native facultative parasites. VII. Some effects of the saprophytes on the bark of poplar and willow on the incidence of *Hypoxylon* canker. *Canad. J. Bot.*, **40** : 61-69.

——————— (1962b) *Ibid.* VIII. Ascospore infection of *Hypoxylon pruniaium* (Klozsch) Cke. *Canad. J. Bot.*, **40** : 897-901.

BORDERS, H. I. (1938) Unpublished M. S. Thesis, University of Minnesota.

BOURRIQUET, G. (1946) Less maladies des plantes cultivees a Madagascar. *Encylopedie mycol.*, **12** : 137-166.

BRIAN, P. W. (1957) *Symposia Soc. Exptl. Biol.*, No. **11** : 166-182.

——— Effects of antibiotics on plants. *Ann. Rev. Plant Physiol.*, **8** : 413-423.

BROWN, R. (1922) Studies in the physiology of parasitism. VIII. On the exosmois of nutrient substances from the host tissue into the infection drop. *Ann. Bot., Lond.*, **36** : 101-119.

——— (1936) The physiology of host parasite relations. *Bot. Rev.*, **2** : 236-281.

BROWN, J. G. AND A. M. BOYLE (1944) *Science*, **100** : 528.

——————— Effect of penicillin on a plant pathogen. *Phytopathology*, **34** : 760-761.

BROWN, J. G. AND D. M. HEEP (1946) *Science*, **104** : 208.

CROWDY, S. H., J. F. GROVE, H. G. HEMMING AND K. C. ROBINSON (1956) *J. Expt. Bot.*, **6** : 42-64.

CRUICKSHANK, I. A. M. (1963) Disease resistance in plants—a review of some recent developments. *J. Aust. Inst. Agric. Sci.*, **29** : 23-30.

CRUICKSHANK, I. A. M. AND DAWN R. PERRIN (1961) Studies on phytoalexins. III. The isolation, assay, and general properties of a phytoalexin from *Pisum sativum* L. *Aust. J. biol. Sci.*, **14** : 336-348.

——————— (1963) VI Pisatin : the effect of some factors on its formation in *Pisum sativum* and the significance of pisatin in disease resistance. *Aust. J. biol. Sci.*, **16** : 111-128.

DICKSON, J. G. (1953) *Rept. Intern. wheat stem rust conf.*, Winnipeg, Jan. 5-7 : 106.

EL-HELALY, A. F. *et al.* (1962) Studies on fungi associated with onion crop in the field and during storage. *Phytopath. Medit.*, **2** : 37-45.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ENGEL, HORST (1939) Das Verhalten der Blätter bei Benetzung mit Wasser. *Jahrb. Wiss. Bot.*, **88** : 816-861.

FARKAS, G. L. AND G. A. LEDINGHAM, (1958) Metabolic aspects of the self-inhibition of stem rust uredospores. (Abstr.) *Phytopathology*, **48** : 392.

FAWCETT, C. H. (1963) Antifungal compounds in plants : some recent developments. Abstracts. *Vth International Pesticides Congress*, London, 17-23 July.

FORSYTH, F. R. (1955) The nature of the inhibiting substance emitted by germinating uredospores of *Puccinia graminis tritici*. *Canad. J. Bot.*, **33** : 363-373.

GAÜMANN, E. (1951) *Pflanzliche Infektionslehrer* (2nd Ed.), Birkhäuser, Basel.

——— (1956) *Experientia*, **12** : 411-418.

GRAY, R. A. (1958) The downward translocation of antibiotics in plants. *Phytopathology*, **48** : 71-78.

HAFIZ, ABDUL (1952) Basis of resistance in gram to *Mycosphaerella* blight. *Phytopathology*, **42** : 422-424.

HILBORN, M. T. (1953) Effect of various chemicals on infection by *Rhizoctonia solani* and *Verticillium albo-atrum*. *Phytopathology*, **43** : 475.

HOYER, H. (1962) Ein Beitrag zur kenntnis der selbsthemmung der uredosporen keimung bei *Puccinia triticina* Erikss. und anderen Rostarten. II. Die Hemmsteffbildung (Abstr.), *Zbf. Bakt.*, **115** : 266-296.

JEROME, S. M. R. AND K. O. MÜLLER (1958) Studies on Phytoalexins. II. Influence of temperature on resistance of *Phaseolus vulgaris* towards *Sclerotinia fructicola* with reference to phytoalexin output. *Aust. J. biol. Sci.*, **2** : 303-314.

JOHNSTON, C. O. and M. D. HUFFMAN (1958) Evidence of local antagonism between two cereal rust fungi. *Phytopathology.*, **48** : 69.

KEENER, P. D. (1954) *Cladosporium aecidiicola* Thuem and *Tuberculina perscina* (D) Sacc. associated with *Puccinia conspicua* Arth. Mains. on *Helenium hoopesii* A. Gray in Arizona. *Plant Dis. Reptr.*, **38** : 693-694.

KLINKOWSKY, M. (1959) Fungistatic antibiotics, especially polyenes, and plant protection. *Recent Advances in Botany*, **1** : 393-400.

KOVACS, A. (1955) über die Ursachen der unterschiedlichen Resistenz der Zuckerrbensorten gegen *Cercospora beticola* Sacc. *Phytopath. Z.*, **24** : 283-298.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

KRASIL, NIKOV, N. A. AND E. A. RAZNITZYNA (1946) A bacterial method of controlling damping-off of Scot pine seedlings caused by *Fusarium*. *Agrobiologiya*, **5** : 109-121.

KUC, J. *et al.* (1956) Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with *Helminthosporium carbonum*. *J. Amer. Chem. Soc.*, **78** : 2123-2125.

LAST, F. T. (1955) Seasonal incidence of *Sporobolomyces* on cereal leaves. *Trans. Brit. mycol. Soc.*, **38** : 221-239.

LEBEN, C. (1964) Influence of bacteria isolated from healthy cucumber leaves on two leaf diseases of cucumber. *Phytopathology*, **54** : 403-408.

LEDINGHAM, R. J. *et al.* (1949) The significance of the normal flora on wheat seed in inoculation studies with *Helminthosporium sativum*. *Proc. Canad. Phytopath. Soc*, **16** : 10-11.

LE ROUX, P. M. AND J. G. DICKSON (1957) Physiologic specialization and genetics of *Puccinia sorghi* on corn and *Puccinia purpurea* on sorghum. *Phytopathology*, **47** : 101-107.

LOESCHKE, V. AND H. BUTIN (1961) On the biological and chemical determination of fungistatic substances in the bark of different poplar varieties. *Meded. Land-bttogesch. Gent.*, **26** : 1548-1552.

MARTIN, J. T. *et al.* (1957) Defence mechanism of plants against fungi. *Nature, Lond.*, **180** : 796-97.

MORTON, D. J. AND G. A. PETERSON (1960) An inverse relationship between the severities of *Helminthosporium* leaf-blade and *Septoria* leaf-sheath symptoms on Barley. *Plant Dis. Reptr.*, **44** : 23-24.

MRIDUL GOEL AND S. SINHA (1961) Effect of antibiotics and sulpha drugs on inhibition of spore germination of certain surface isolates from vegetable seeds. *Proc. 48th Indian Science Congress Association, Part III (Abstr)* : *251*.

MÜLLER, K. O., (1956) *Phytopath. Z.*, **27** : 237-254.

MÜLLER, K. O., (1958) Studies on Phytoalexins. I. The formation and the immunological significance of phytoalexin produced by *Phaseolus vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytophthora infestans*. *Aust. J. biol. Sci.*, **2** : 275-300.

NIKITINA, MME. K. V., (1961) The phytoncidal action of apple and pear. pp. 143-147, Sverdlovsk.

NOVOGRUDSKI, D., (1937) The use of microorganisms in the control of fungal diseases of cultivated plants. *Bull. Acad. Sci. Biol. U.R.S.S., Ser.*, **1** : 273-293.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PETRUSHOVA Mme. N. I. AND Mme. N. B. MESCHCHANINOVA (1901) Fungicidal properties of plants from the family Ranunculaceae. *Trud. nikitsk. bot. Sada.*, **33** : 247-252.

PON. D. S., C. E. TOWNSEND, G. E. WESSOMAN, C. G. SCHMIDT AND C. H. KINGSOLVER (1954) A *Xanthomonas* parasitic on uredia of cereal rusts. *Phytopathology*. **44** : 707-710.

PRAMER, D. (1959) Eradicant and therapeutic materials for disease control. Antibiotics. *Recent advances in Botany*, **1** : 452-456.

PRESCOTT, G. C., H. EMERSON AND J. H. FORD (1956) *J. Agric. Food Chem.*, **4** : 343-345.

RUBENCHYK, L. T., V. A. KORDYM AND S. T. CHERNYKH (1962) Rozvytok nikroorganizmiv u filosferi deyakykh roslyn V. pryrodnykh umovakh. *J. Microbiol.*, (Kiev), **24** : 3-7.

RUINEN, JAKOBA. (1961) The phyllosphere. I. An ecologically neglected millieu. *Plant and soil*, **15** : 81-109.

SALLANS, B. J. *et al.* (1949) Testing wheat seedlings for resistance to *Helminthosporium sativum.* with reference to antibiosis. *Proc. Canad. Phytopath. Soc.*, **16** : 11.

SANWAL, B. D., (1956) *Phytopath. Z.*, **25** : 333-384.

SIMARD, J. *et. al.* (1958) Screening of microorganisms inhabiting apple leaf for their antibiotic properties against *Venturia inaequalis* (Cke) Wint. *Rept. Quebec Soc. Prot. Pl.*, **39** : 59-67.

SIMARDS, P. M. (1947) The influence of antibiosis in the pathogenecity of *Helminthosporium sativum. Sci. Agric.*, **27** : 625-632.

SINGH, N. P , AND S. SINHA (1962) Microorganisms of some vegetable surfaces and tissues and their antagonisms. *Proc. 49th Indian Sci. Cong. Association, Part III (Abstr.)* : 252.

TIMONIN, M. I. (1962) Antifungal activity of pentatomid scent glands. *Canad. J. Microbiol.*, **8** : 594-595.

TOKIN, B. P. (1960) *Guliteli microbov-fitoutsidy.* (*Phytoncides, the destroyers of microbes*) 198 pp., Moscow Izdat. Sov. Rossiya.

TOPPS, J. H. AND R. L. WAIN (1957) Fungistatic properties of leaf exudates. *Nature, Lond.*, **179** : 652-653.

TUBEUF,C. VON (1930) *Z. Pflanzenkrankh.*, **40** : 177-181.

TVEIT, M. AND R. K. S. WOOD (1955) The control of *Fusarium* blight in oat seedlings with antagonistic species of *Chaetomium. Ann. appl. Biol.*, **43** : 538-52.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TYNER, L. E. AND BARBARA A. MCKINNON (1964) Fungi of barley seeds and their associative effects. *Phytopathology*, **54** : 506-508.

UEHARA, K. (1958) On some properties of phytoalexin produced as a result of the interaction between pea (*Pisum sativum*) and *Ascochyta pisi*. I. On the activity as affected by ultraviolet irradiation and on some physicochemical properties of phytoalexin. *Ann. Phytopath. Soc. Japan*, **23** : 230-234.

UEHARA, K. (1959) On phytoalexin produced as the result of the interaction between Pea (*Pisum sativum L.*) and *Ascochyta pisi* Lib. II. The effect of the duration of spore suspension inoculations and preliminary treatment of pea pods with ether or heat on the production of phytoalexin. *Ann. Phytopath. Soc. Japan*, **25** : 85-91.

UEHARA, K. (1952) Identification and estimation of phytoalexin produced by pea pods by means of ultraviolet absorption spectrum. *Bull. Hiroshima Agric. Coll.*, **2** : 1-7.

URITANI, F. AND T. AKAZAWA (1955) *Science*, **121** : 216-217.

VAN VELSEN, R. J. (1957) A study of the pathogenicity of *Helminthosporium* spp. (B.Ag.Sc. Hons. Thesis, University af Adelaide).

VÖRÖS, J., Z. KIRALY AND G. L. FARKAS (1957) *Science*, **126** : 1178-1179.

VOZNYAKOVSKAYA, MME YU. M. AND YU. G. SHIROKOV (1961) The use of epiphytic mycoflora in the control of grey mould of strawberry. pp. 170-179, *Erevan, Acad. Sci. Armenian U.S.S.R.*

WEINDLING, R. (1932) *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*, **22** : 837-845.

WEINDLING, R. (1934) Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology*, **24** : 1153-1179.

WEINDLING, R. AND O. H. EMERSON (1936) The isolation of a toxic substance from the culture filtrate of *Trichoderma*. *Phytopathalogy*, **26** : 1068-1070.

WIBE, O. AND DONALD I. MORTON (1962) Inhibition of *Septoria passerinii* development in excised barley leaves by *Helminthosporium sativum* and by cell free filtrates. *Phytopathology*, **52** : 373.

WILSON, E. M. (1958) Aspartic and glutamic acids as self-inhibitors of uredspore germination. *Phytopathology*, **48** : 595-600.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

WOOD, R. K. S. (1951) The control of diseases of lettuce by the use of antagonistic organisms. The control of *Botrytis cinerea* Pers. *Ann. appl. Biol.*, **38** : 203-230.

WRIGHT, J. M. (1956) The production of antibiotics in soil. IV. Production of antibiotics in coats of seed sown in soil. *Ann. appl. Biol.*, **44** : 561-556.

YARWOOD, C. E. (1954) Mechanism of acquired immunity to a plant rust. *Proc. Nat. Acad. Sci. Wash.*, **40** : 374-377.

YARWOOD, C. E. (1956) Simultanous self-stimulation and self-inhibition of uredospore germination. *Mycologia*, **48** : 20-24.

ZAUMEYER, W. J., H. R. MITCHELL AND H. H. FISHER (1953) Field control of halo blight of beans with streptomycin. *Phytopathology*, **43** : 407.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CARBON REQUIREMENTS OF COLLETOTRICHUM INAMDARII

S. K. HASIJA

(Accepted for publication November 26, 1964)

Colletotrichum inamdarii Lal causing anthracnose of *Carissa carandas* L., was first reported by Lal and Singh (1953) from Uttar Pradesh. Agarwal (1962) reported *Gloeosporium carissae* Agarwal on the same host causing leaf spot and dieback disease from Jabalpur (M. P.), during 1958. The author, however, came across and isolated *C. inamdarii* on the same host at Jabalpur during September 1962. Since the disease reaction in plants is the resultant of the metabolic activities of the parasite, it is essential to make a thorough study of the physiology of the parasite.

The present paper deals with the utilization of various carbon compounds by *C. inamdarii*.

MATERIAL AND METHODS : Isolations were made from the infected leaves of *Carissa carandas* and single spore cultures obtained. Asthana and Hawker's medium 'A' containing KNO_3 - 3.5 gms., KH_2PO_4 – 1.75 gms., $MgSO_4$ $7H_2O$ – 0.75 gms., Glucose – 5 gms., in one litre of distilled water, was used as the basal medium. Glucose of the basal medium was substituted by different carbon compounds singly and care was taken to include the same amount of carbon as present in 5 gms. of glucose per litre. Purest available chemicals and glass wares were employed during the present studies. 50 ml. of the basal media was taken in 150 ml. conical flasks which were steam sterilized at 15 lb. pressure for 15 minutes. The pH of the media was adjusted to 5.3 before autoclaving by adding NaOH or HCl. The inoculation was done by taking inocula of equal size, cut by agar disc method. After inoculation the flasks were incubated at room temperature for 15 days.

The filtrates from each carbon compound were chromatographically analysed daily for 15 days to detect the presence of sugar in the media. The method described by Ranjan, Govindjee and Laloriya (1955) was employed for these studies. The chromatograms were run in *n*-butanol, acetic acid and water (4 : 1 : 5) for 20 hours. They were then dried at room temperature for 2 hours and were later developed in aniline diphenylaminephosphate. Finally the chromatogrames were dried and placed in an electric oven at 70°C for 3 minutes. The average R*f* values of various sugars were determined. The average dry weight of four replicates and the final pH of the media have been recorded.

RESULTS : The best growth of the fungus was obtained on glucose followed by mannose, raffinose, fructose, sucrose, maltose, lactose, rhamnose, galactose, arabinose and xylose. Sporulation was excellent on mannose, xylose and maltose; very good on glucose, fructose and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

arabinose; good on sucrose and galactose; moderate on lactose; fair on rhamnose while poor on raffinose.

TABLE 1 : Dry weight in mg., sporulation and final pH of *C. inamdarii* on various carbon compounds. (Monosaccharides)

Sugars	Final pH	Rg value	Dry wt.	Sporulation	Day of utilization
Glucose	7.7	1	176	Very good	4
Fructose	8.1	1.05	140	Very good	5
Galactose	7.1	0.91	103	Good	5
Mannose	7.5	1.04	148	Excellent	5
Arabinose	7.8	1.07	071	Very good	7
Rhamnose	7.7	1.08	110	Fair	5
Xylose	8.2	1.12	070	Excellent	5

TABLE 2 : Dry wt. in mgs., sporulation, pH, time of appearance of hydrolytic products in the medium and their rate of utilization by *C. inamdarii* on di-and trisaccharides.

Sugars	final pH	Rf value	Hydrolytic products.	Time		Dry wt.	Sporulation.
				Day appeared	Day utilized		
Sucrose	7.4	0.80	Glucose	5	7	140	Good
			Fructose	5	8		
Maltose	7.5	0.77	Glucose	6	10	125	Excellent
Lactose	7.3	0.70	No hydrolytic product was detected.		12	122	Moderate
Raffinose	7.9	0.55	Sucrose	5	8	144	Poor
			Galactose	5	7		

It is evident from Table 1 that glucose served as the best source of carbon amongst monosaccharides and was completely utilized on the 4th day. Mannose, rhamnose, fructose and galactose were also

CC-0: In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

satisfactory sources of carbon whereas arabinose and xylose proved to be of poor source. All the monosaccharides were utilized on the 5th day except the arabinose which was utilized on the 7th day.

Daily chromatographic analysis on the utilization of di- and trisaccharides as given in Table 2 revealed, that sucrose is utilized through hydrolytic pathway. It was observed that on the 5th day the entire sucrose was converted into glucose and fructose and it totally lost its identity from the medium. Both these hydrolytic products, glucose and fructose. were completely consumed by 7th and 8th day respectively.

Maltose was converted into glucose on the 6th day which was, in turn, fully consumed by the 10th day. Lactose was not hydrolysed by this fungus and its hydrolytic products, glucose and galactose, were not detected on the chromatogram. Lactose was fully utilized by the 12th day. Raffinose was also utilized through hydrolytic pathway. On the 5th day only two sugars were detected on breakdown *viz.*, sucrose and galactose. Galactose was fully consumed by the 7th day, while sucrose (whose further breakdown into glucose and fructose could not be traced) was consumed by the 8th day.

DISCUSSION : The results of the present studies show that *Colletotrichum inamdarii* can utilize carbon from different sources fairly well.

Monosaccharides are the most easily assimilable carbohydrates and their utilization is assumed to be direct. Glucose has been found to be the best source of carbon for *C. inamdarii*. Mannose, fructose, rhamnose. and galactose were also satisfactory sources while arabinose and xylose proved to be of poorer sources of carbon.

Glucose is better utilized by most fungi than any other sugar and is nearly universal carbon source. (Agarwal, 1957, 1962; Ganguli, 1959 ; Mehrotra, 1951 and Tandon and Chandra, 1962). However some fungi are unable to utilize glucose (Sahade, 1940). Rhamnose has been reported to be a poor source of carbon for *Pestalotia banksiana* and *P. citri* (Tandon and Bilgrami, 1958) but it has been found to be a good source of carbon for *C. inamdarii*. Arabinose and xylose are poor carbon sources for the present fungus. It has also been reported to be a poor source for *C. gloeosporioides* (Tandon and Chandra, 1962).

It was established in the present investigation that the *C. inamdarii* utilizes di- and trisaccharides through the hydrolytic pathway as reported by several investigators (Lilly and Bar nett, 1953 ; Tandon and Bilgrami. 1957, 1958; Tandon and Chandra. 1962 and Agarwal, 1962). Among them, raffinose and sucrose were found to be of good sources of carbon.

Raffinose on complete hydrolysis yields equal amount of glucose. fructose and galactose. Cochrane (1958) stated that raffinose, if attacked at one linkage yields galactose and sucrose and attacked at the other yields fructose and melibiose. In the present studies sucrose and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

galactose were detected as the hydrolytic products. Some investigators found only fructose and melibiose as the result of breakdown of raffinose (Tandon and Bilgrami, 1958). Synthesis of two oligosaccharides along with glucose and fructose has also been reported, during the utilization of sucrose, by *Colletotrichum gloeosporiodes*. It was found that out of the two hydrolytic products, glucose was utilized earlier than fructose. Similar behaviour has been reported with *Phyllosticta* (Tandon and Bilgrami, 1957).

Maltose was hydrolysed into glucose. On comparing the dry weight on glucose alone, it is clear that the fungus grows better on glucose than maltose. Similar results have been obtained by other workers, (Agarwal and Shinkhede 1959; Ganguli, 1959; Tandon and Chandra, 1962), whereas some have obtained better growth on maltose than glucose with their organisms. Tandon and Bilgrami (1957) attributed the results to the presence of oligosaccharides synthesized during assimilation. No such oligosaccharides could be detected during present studies. During the utilization of lactose no hydrolytic pathway could be detected. It is possible that the hydrolytic products might have been utilized simultaneously and as such it could not be detected on the chromatogram.

The final pH of all the cultural media was changed towards alkalinity due to the metabolic activity of the fungus and it ranged between pH 7.1 to 8.2. It is quite obvious from the results that the final pH of the medium did not have any marked effect on the growth and sporulation of *C. inamdarii*.

SUMMARY

The utilization of various carbohydrates by *Colletotrichum inamdarii* has been studied. Circular paper chromatography was employed for the daily analysis, to find out the utilization of carbohydrates, and their hydrolytic products. Amongst monosaccharides, glucose was the best source of carbon followed by mannose, fructose, rhamnose and galactose. Arabinose and xylose were of poor source. Raffinose and sucrose were found to be the best source amongst di- and trisaccharides, followed by maltose and lactose. Daily utilization and hydrolytic pathway of these sugars could be traced in the medium. The hydrolytic products of lactose could not be traced. The final pH of all the carbohydrates was towards alkalinity and ranged between pH 7.1 to 8.2. It had no marked influence on the growth and sporulation of *C. inamdarii*.

ACKNOWLEDGEMENTS ; The author expresses his grateful thanks to Dr. G.P. Agarwal, Department of Botany, Govt. Science College, Jabalpur for his valuable guidance and encouragements. Thanks are also due to the Principal, Govt. Science College, Jabalpur for providing laboratory facilities.

Department of Botany,
Govt. Science College,
Jabalpur (M. P.).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

AGARWAL, G. P. (1957) Effect of different carbon compounds on the growth and sporulation of *Fusarium coeruleum* (Lib.) Sacc. *Proc. Nat. Acad. Sci. India* **27** (B) : 332-340.

AGARWAL, G. P. (1962) Anthracnose disease of 'Karaunda' caused by *Gloeosporium carissae. Proc. Nat. Acad. Sci. India,* (B) **32** : 335-344.

————AND G. C. SHINKHEDE (1959) Pysiological studies on *Helminthosporium rostratum. Phyton,* **13** : 45-54.

COCHRANE, V. W. (1958) *Physiology of fungi,* John Wiley & Sons, New York.

GANGULI, S (1959) *Studies on* Pestalotia sp. M. Sc. Thesis, Univ. of Jabalpur.

LAL, A AND R. P. SINGH (1953) Anthracnose disease of *Carissa carandas* caused by *Colletotrichum inamdarii. J. Indian. bot. Soc.,* **32** : 54-63.

LILLY, V. G. AND H. L. BARNETT (1953) The utilization of sugars by fungi. *West Virginia Univ. Agric. Expt. Sta. Bull.* 38 pp.

MEHROTRA, B. S. (1951) Physiological studies of some Phytophthoras. III. Carbon requirements. *Lloydia,* **14** : 122-128.

RAMAKRISHANAN, T. S. (1941) Studies on the genus *Colletotrichum.* Physiological studies on *Colletotrichum. Proc. Indian Acad. Sci.,* **14** : 395.

RANJAN, S., GOVINDJEE AND M.M. LOLORIYA (1955) Chromatographic studies on the amino acid metabolism of healthy and diseased leaves of *Croton sparsiflorus* Morong. *Proc. Nat. Acad. Sci. India,* **21** : 42-47.

SCHADE, A. L. (1940) The nutrition of *Leptomitus. Amer. J. Bot.,* **27** : 376-384.

TANDON, R. N. AND K. S. BILGRAMI (1957) Assimilation of disaccharides by some fungi causing leafspot disease *Proc. Nat. Acad. Sci. India,* **46** : 274-284.

————AND————(1958) The utilization of monosaccharides by *Pestalotia banksiana* and *P. citri. Proc. Nat. Acad. Sci. India,* **25** : 138-142.

————AND S. CHANDRA (1962) Nutrition of *Colletotrichum gloeosporioides. Mycopath. et. Mycol. Appl.,* **18** : 213-224.

THIND, K. S. AND H. S. RANDHAWA (1957) Studies on nutrition of fungi I. *Proc. Nat. Acad. Sci. India,* **27** (B) : 39-46.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ON TWO UNRECORDED RACES AND A NEW BIOTYPE OF PUCCINIA RECONDITA

M. M. PAYAK

(Accepted for publication November 28, 1964)

Brown rust is one of the important limiting factors in wheat culture in India. Of late, for reasons not yet well understood, the incidence of this rust seems to be on the increase. Sampling for the occurrence of physiologic races as well as an analysis of their frequency has been in progress for over 30 years. Unrecorded races continue to turn up even from fairly well-surveyed localities. This may be due to the increase in efficiency of the sampling technique (*e.g.*, establishment of rust nurseries at selected sites in the wheat growing regions which function as "living traps"). It could also be due to the growing of wheat varieties having a varied germ plasm or perhaps some factors connected with the life-cycle of the rust.

So far following races have been recorded from this country: 10, 11, 17, 20, 26, 63, 70, 77, 106, 107, 108 and 162.

Race 131 : During 1959-60 crop season a race turned up in almost pure form (without any admixture with any other race) in a collection made on the differential *Brevit*. Isolations taken from *Brevit* as well as the stock collection showed identical reactions with those of race 131 as given in the International Register (Johnston and Levine[1]; Johnston[2]). These have been detailed in Table 1.

Basile[3] proposed a unified numeration scheme for the leaf rust races. The scheme is based on reactions as expressed on 5 differentials only. These are : *Malakof*, *Webster*, *Loros*, *Mediterranean*, and *Democrat*. In accordance with this scheme, the present race 131 belongs to race group 10 in which previously known Indian races like 11 and 26 are also grouped.

It is noteworthy that this is the second race which has been isolated on a differential cultivar grown in the rust nursery. The first one which was so picked up is race 77. It was found on *Mediterranean* at Pusa during the crop year 1954-55.

Race 16 : A collection of Brown rust (1958-59 crop) from Wellington (Nilgiris, Madras) on the wheat variety, *Portugal-24*, was inoculated on *Agra local* wheat (a control or check variety) for establishment of the culture and subsequent multiplication. Surprisingly, resistant

CC-0, In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Reactions of races 16, 131 (Indian material) compared with those given in the International Register and of 162 and 162-A (new Biotype)

Locality, host, stock and/or isolation	International Differentials									Race	Unified race group
	Mal	Car	Brev	Web	Lor	Med	Huss	Dem	Thw.		
Poona Brevit	0;	4	4	0;-1	4	0;	0;	0;	4	131	
Brevit (4) Single-spored	0;	4	4	0;-1	4	0;	0;	0;	4	131	10
U. S. A. (Johnston[1])	0;	3	3	2	3	0;	2+	0;	—	131	10
Wellington, Port-24, Agra local (0;-2) Single spored	0;	1-2	1-2	0;-1	2+	0;-1	0:	0;	0;	16	1
Iarkina (Russia) (Johnston[2])	2	2	2	2	2	1	2	1	R ?	16	1
Gwalior NP 720 Malakof (2)	0;	4	4	3	4	4	X	4	0;-2	162	17
Rithani, U.P. Local Wheat Thew (4)	0;	4	4	3	4	4	X	4	4	**162-A**	

type of infection was observed. Isolations from this resistant type of infection were increased on *Khapli* (*Triticum diccocum*)—a variety commonly grown in the Nilgiris—after having ascertained that it is susceptible to this isolation.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The reactions produced on the differentials agreed with those of race 16 as given in the International Register. Race 1 also produces similar reactions but as pointed out by Johnston and Levine[1] and Johnston[2], these two races are separable by the variety *Thew*. Reactions of the Indian isolate as well as those given in the International Register have been set out in Table 1. In the scheme proposed by Basile[3], this race is included in Race Group 1 in which is also placed race 63 which was first collected from India in 1933.

This is the first record of a brown rust race which produces resistant type of infection on *Agra local*. It indicates the need for selecting a better control variety or at least to have an additional one which could be used in conjunction with *Agra local*.

New Biotype of Race 162 : During the crop year, 1959-60, a collection of brown rust on a local wheat variety was received from Rithani, U. P. The collection, when inoculated on the differential set showed reactions of race 162 except on *Thew*. This Australian variety is being used in the analysis work in India as a supplementary or auxiliary differential. As given in Table 1, the original Indian isolate of race 162 produces resistant type of infection on *Thew* while with the Rithani isolate it has been found to be susceptible. It has, therefore, been recognized as a new biotype and is being designated as **162-A**.

On *Carina* and *Brevit* the type race as well as the new biotype produce a mildly susceptible type of reaction which under some conditions resembles a mixed type (2—3). The X type of reaction on *Hussar* also under conditions of high temperature turns into a susceptible type.

SUMMARY

Race 16 (unified race group 1) and 131 (unified race group 10) of leaf rust of wheat have been recorded from India, the former from Wellington, Nilgiris and the latter from Poona. It is noteworthy that with race 16 the commonly used control variety (Agra local) is resistant but Khapli is susceptible. The reactions of the Indian isolates have been compared with those given in the International Register. A new biotype of race 162 designated as **162-A** has been met with in a collection made at Rithani, U. P. (1959-60 crop). On the auxiliary differential *Thew*, with this biotype, susceptible type of reaction is produced. With the original isolate of race 162, on the other hand, resistant reaction is obtained.

ACKNOWLEDGMENT : I am grateful to Drs. B. L. Chona, and R. Prasada for their valuable suggestions.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute,
New Delhi.

---

[1]Basile, R. *Plant dis. Reptr.*, 41 : 508-511, (1957).
[2]Johnston, C. O. and M. N. Levine, *Plant dis. Reptr. Suppl.*, **233** : 104-120, (1955).
[3]Johnston, C. O. *Crops Research* 15 pp., (1961)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A NEW RUST ON SCHIZANDRA GRANDIFLORA

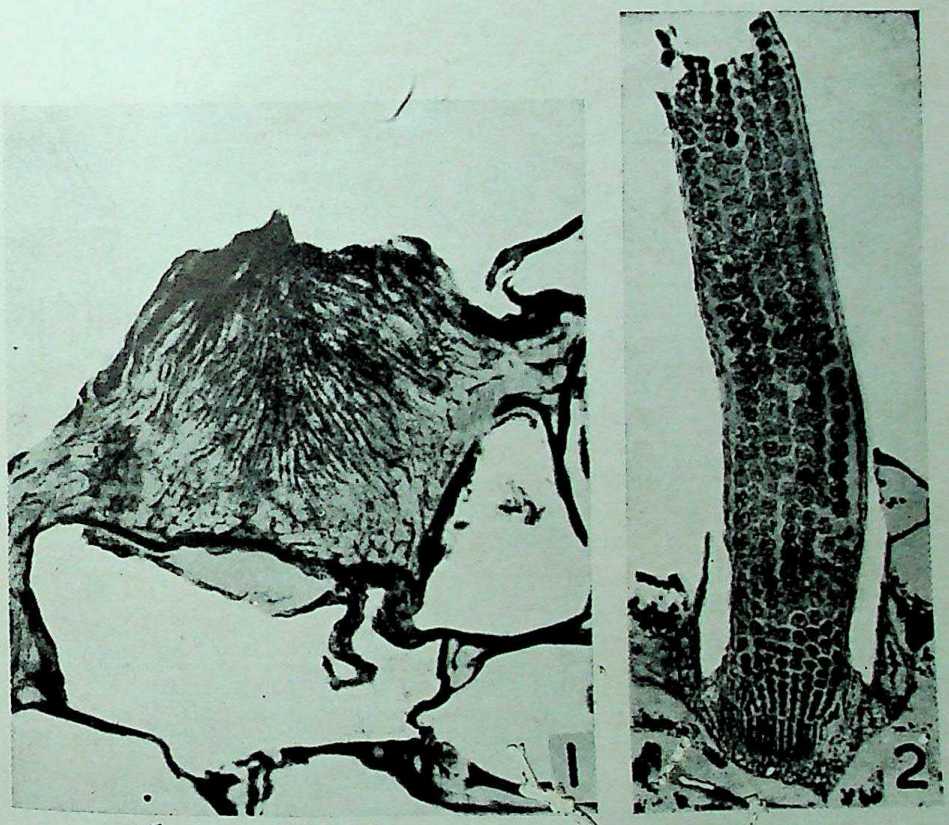
HARDEV SINGH AND S. JALAN*

(Accepted for publication November 30, 1964)

While studying the structure of the fruits of *Schizandra*, some of them were found to be infected with a rust fungus. They showed gregarious 'aecial cups' projecting from the centre of a discoloured area (Fig. 3). On sectioning them it was found that the peridium is well developed and the two nuclei in the 'aeciospores' fuse. The 'aeciospore' is therefore called a teliospore in the following pages.

The pycnia occur in the centre of the colony of telia. In the collections available to us, the pycnia had passed their active life. They are flask-shaped and subepidermal (Fig. 1). In all probability, they lack periphyses.

The telium is narrow and tumbler-shaped in longisection (Fig. 2). It projects 400 μ from the host tissue and has a diameter of 133 μ The total length of the telium is around 680 μ. Occasionally, a few short

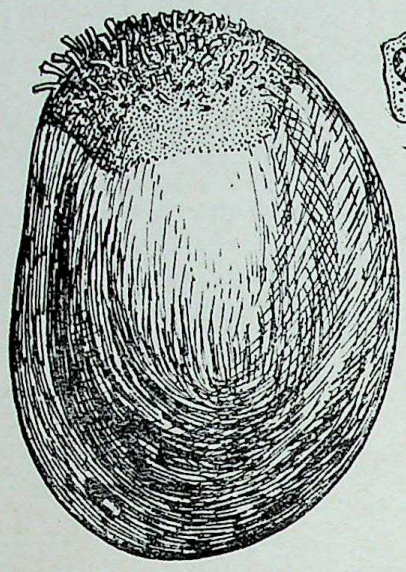


Figs. 1—2. 1. Longisection of a pycnium. × 400. 2. Longisection of a telium. × 120.

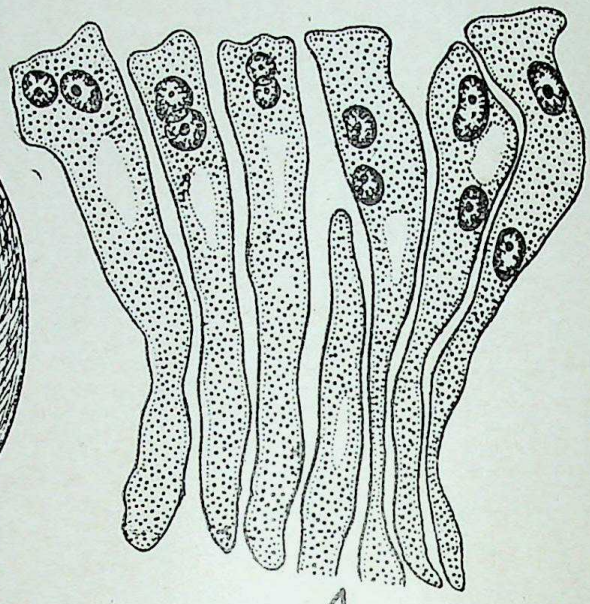
*Present Address : Department of Botany, University of Rajasthan, Jaipur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

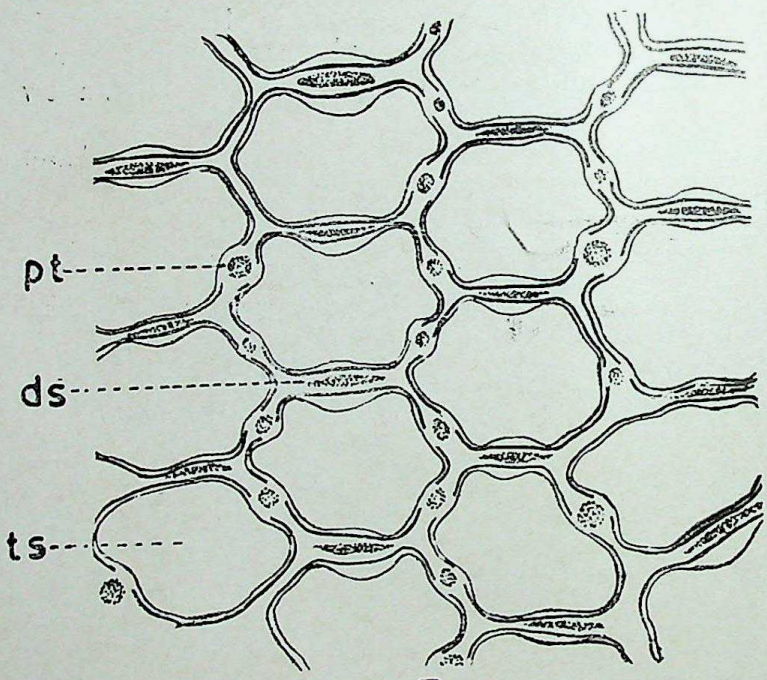
Digitized by Arya Samaj Foundation Chennai and eGangotri



3



4



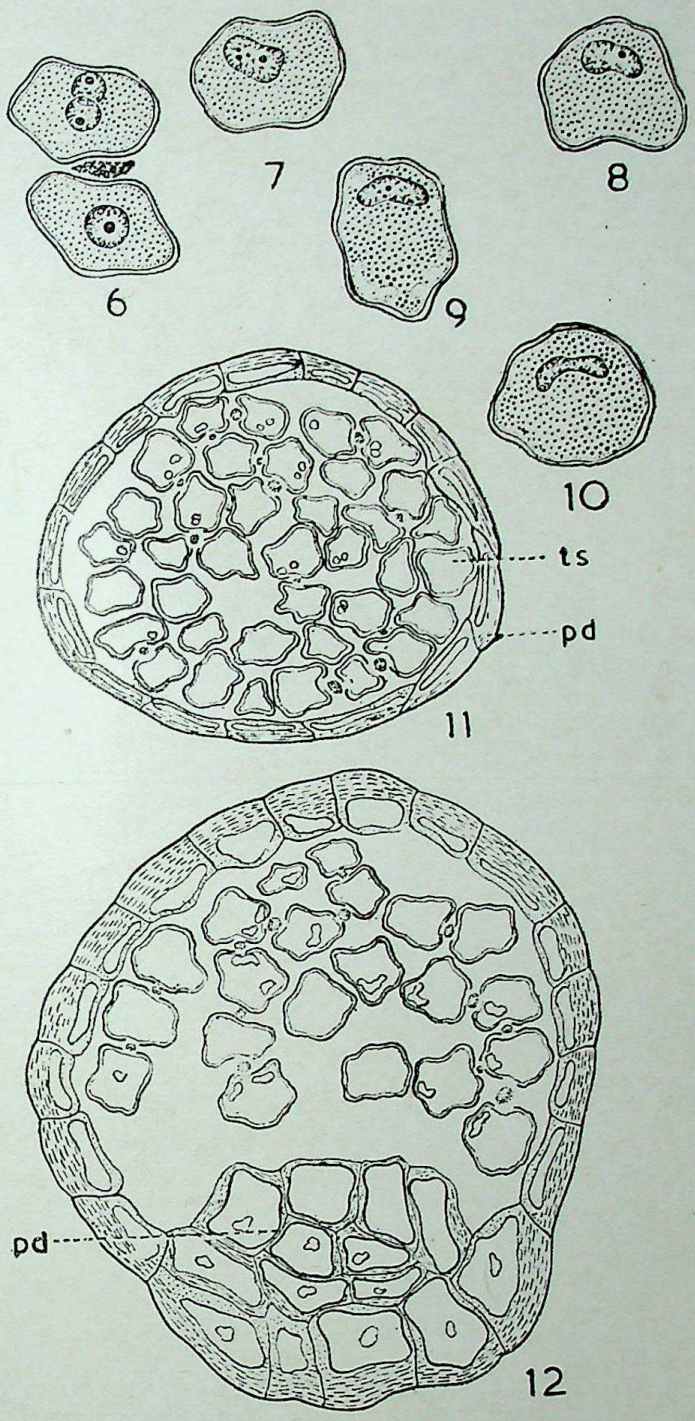
5

Figs. 3—5. (Abbreviations : *ds*, disjunctor ; *pd*, peridium ; *pt*, pore thcikening ; *ts*, teliospore). 3. Maturing fruitlet of *Schizandra grandiflora* showing a colony of a telia. × 5. 4. Some of the based cells of the telium × 700. 5. Part of teliospore chains : disjunctors and pore thickenings are prominent. × 700.

telia were seen to project into the ovarian cavity. There is a narrow space between the telium and the host tissue (Fig. 2). Remnants of the displacement zone of the 'protoaecium' can be made out in the mature telium (Fig. 2). The basal cells of the telium are very long (40 μ), vacuolate and two-nucleate, the two nuclei being situated mostly near the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 6—12. 6—10. Teliospores in various stages of development. ×485. 11. T. s. telium, the peridium is one layered. ×230. 12. Same, the peridium is several layered in the lower region. ×230.

lower end of the cell (Fig 4). The teliospores lie in longitudinal rows and are separated from each other by disjunctor cells (Fig. 5). The chains of teliospores are so arranged that the angularities of the adjacent rows fit into each other. The spores show prominent pore-thickenings

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Fig. 5). According to Dodge[2] they greatly aid the expulsion of spores from the aecium. A mature teliospore is angular and isodiametric, measures around 20 μ across, and shows a thin and verrucose wall. When just formed, the teliospore is binucleate but the two nuclei soon fuse (Fig. 6). The fusion nucleus moves to the periphery of the spore and becomes greatly elongated in a tangential direction (Figs. 7-10). Its chromatin becomes compact and the nucleolus does not remain so distinct.

The peridium is persistent and comprises a single layer of cells which closely adhere to each other (Fig. 11). Occasionally the peridium is several-layered at certain points (Fig. 12). The peridial cells are thick-walled, the outer tangential walls being especially thick (Fig. 11).

According to Cummins[1] aecia-like telia occur in *Dietelia*, *Endophylloides*, *Endophyllum*, *Kunkelia* and *Pucciniosira*. The rust described here also shows aecidioid telia. The present fungus is distinct from *Pucciniosira* which has two-celled teliospores; from *Dietelia* which lacks disjunctors between the teliospores; from *Endophylloides* in having non-adhering teliospores and from *Kunkelia* which lacks a peridium. It resembles *Endophyllum* in having typical aecidioid telia with a well developed peridium and non-adhering teliospores (Thirumalachar and Mundkur[3]). A new species of *Endophyllum*—*E. maheshwarii*, after our teacher Prof. P. Maheshwari - is proposed to accommodate this fungus. The specimen has been deposited in the *Herbarium Cryptogamiae Indiae Orientalis*, New Delhi; accession number : H. C. I. O. 28581.

Endophyllum maheshwarii sp. nov.

Pycnia subepidermalia urceolata. Telia aecidioidea, gregaria ad medium arearum colore privatarum in facie fructuum maturantium. Longitudo telii totalis ca. 680 μ, diam. 133 μ; cellulae basales telii ca. 40 μ longae. Disjunctores adsunt. Pori crassitudines manifestae in teliosporis. Teliospora matura verrucosa, ca. 20 μ. diam. Eius vero nucleus elongatus, ad peripheriam jacens. Peridium uniseriatum, continens cellulas parietibus crassis praeditas et arcte adhaerentes.

In foliis viventibus *Schizandrae grandiflora* Hook. f. & Thom. Lect. ad Rambara, Gharwal, a M. A. Rau, mense Septembra, 1958.

ACKNOWLEDGEMENTS : We are grateful to Dr. M. A. Rau, Regional Botanist, Northern Circle, Botanical Survey of India, Dehra Dun, for sending us some fruits of *Schizandra grandiflora*, collected by him from Rambara (situated at 9000 ft. above sea level in Gharwal, W. Himalayas) in September 1958. Further collections were made by one of us (SJ) in August 1959. We are grateful to Prof. P. Maheshwari for interest and encouragement. We are thankful to Prof. G. B. Cummins (Indiana, USA) for supplying data on *Endophyllum*, to Dr. M. M. Payak for critical comments and to Rev. Fr. Dr. H. Santapau for the latin diagnosis.

Department of Botany,
University of Delhi,
Delhi-17.

[1] Cummins, G. B. *Illustrated Genera of Rust Fungi* 1 131 pp. (1964).
[2] Dodge, B. O., *J. agric Res.*, 28 : 923 - 920. 1924).
[3] Thirumalachar, M. J. and Mundkur, B. B. *Indian Phytopath.*, 2 : 193—244, (1949).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SEED MICROFLORA I. SEED-BORNE FUNGI OF RAGI (ELEUSINE CORACANA GAERTN.) THEIR DISTRIBUTION AND CONTROL

J. S. GREWAL AND MAHENDRA PAL

(Accepted for publication November 30, 1964)

Ragi or finger millet (*Eleusine coracana* Gaertn.) is one of the hardiest crops grown in hilly areas of North and North East India as well as in parts of Orissa, Madras, Andhra Pradesh, Mysore, Kerala and Maharashtra and the area under this crop is 5.7 million acres. In spite of such large acreage the annual yield is only 1.7 million tons. Of the several factors that are responsible for lower yields, diseases occupy, undoubtedly, an important place. The chief diseases of *ragi* are blast caused by *Piricularia oryzae* Cav. and blight caused by *Helminthosporium nodulosum* Berk. & Curt. which are known to cause considerable losses.

A large number of *ragi* varieties collected from different States of India were obtained from Botany Division, Indian Agricultural Research Institute, New Delhi, with a view to assess the distribution and incidence of seed-borne fungi. In addition. comparative efficacy of some of the new fungicides against seed-borne fungal pathogens of *ragi* was also tested so that the most effective one may be determined and recommended to the cultivators.

EXPERIMENTAL : Standard International Rules for Seed Testing[1] were followed throughout these studies. From each sample, 200 seeds were vigorously shaken in a test tube containing distilled water and a trace of wetting agent (teepol). The water was removed, centrifuged and examined under the microscope. Seeds were also tested by the agar plate and blotter methods. Four hundred surface-sterilized and unsterilized seeds of each sample were plated in 9.5 cm. petriplates containing potato dextrose agar or sterilized moist blotting paper in each case and incubated at 25 – 28°C. Sterilization of seeds was carried out by dipping the seeds in 0.1 per cent aqueous mercuric chloride for 2-3 minutes after which they were washed in several changes of sterile distilled water.

Seed-borne fungi and their distribution : Centrifuged seed-washings revealed the presence of spores of *Helminthosporium nodulosum* and species of *Fusarium*, *Aspergillus*, *Alternaria* and *Curvularia*. In the agar-plate method, where seeds were placed on the agar medium, the last three fungi overgrew and consequently masked *H. nodulosum* and *Fusarium* sp. and hence it was not possible to study the pathogenic role of first

[1]International rules for seed testing. *Proc. Int. Seed Test. ASS.* **24** : 565-577 (1959).

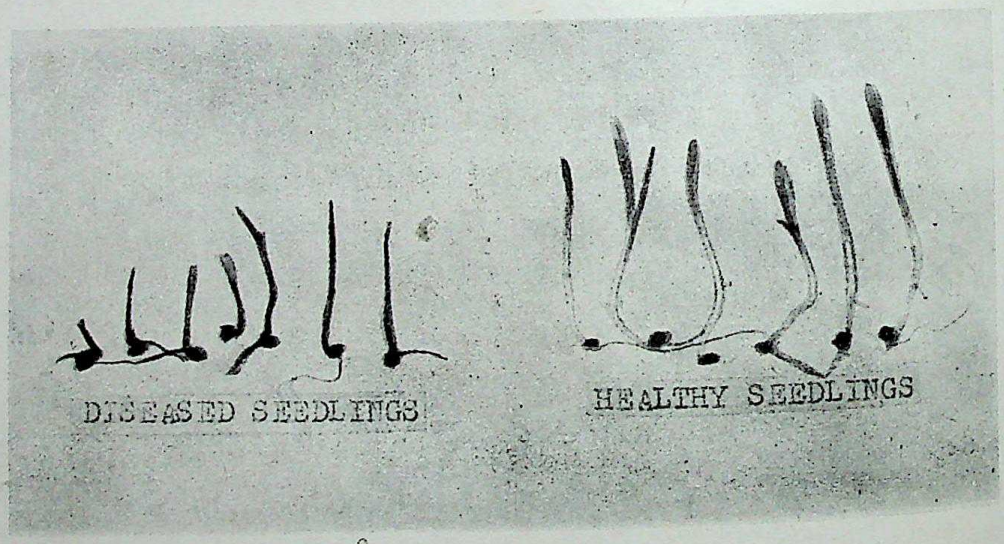
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

two fungi by this method. On the sterilized moist blotters, however, they grew well on the seeds while the three saprophytic fungi grew less vigorously. The blotter method was, therefore, used subsequently for the rapid detection of these pathogens. It was observed that *H. nodulosum* caused seed-rot as well as seedling blight whereas *Fusarium* sp. was found to be responsible for only seed-rot. Infection of *H. nodulosum* was observed to be externally seed-borne in majority of the cases but it was also isolated from the surface-sterilized seeds of variety Madua (1. E. 246) indicating, thereby, that the infection can also be internal. But such deep-seated infection was, however, not found to be more than 9.2 per cent.

The affected seeds and their glumes showed black spots on them which were found to be caused by *H. nodulosum*. *Fusarium* sp. did not cause any clear cut macroscopic symptoms. Both *H. nodulosum* and *Fusarium* sp. emerged from the infected seeds within 2 to 3 days at 25–28°C when they were allowed to germinate on sterilized moist blotters. Pinkish white colonies of *Fusarium* sp. and grey to dark grey colonies of *H. nodulosum* could be readily distinguished on the seed surface. All the seeds affected with *Fusarium* sp. and some of the seeds infected with *H. nodulosum* failed to germinate while the remaining seeds infected with *H. nodulosum* produced very weak seedlings with dark brown lesions on their coleoptiles and roots (Fig. 1). The diseased seedlings were killed after a few days.

Fig. 1



Seeds of thirteen varieties of *ragi* obtained from seven different states of India were tested in these studies. The data obtained are recorded in Table 1.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Incidence and distribution of seed-borne pathogens in different varieties of *ragi*.

Ragi varieties	Place of collection	Percentage infection *Fusarium* sp.		*H. nodulosum*		Total Loss
		Seed rot	Seed-ling blight	Seed rot	Seed-ling blight	
Madua (I. E. 246)	Bihar	3.0	nil	13.0	32.5	48.5
Madua (I. E. 137)	Bihar	Nil	"	Nil	34.0	34.0
Madia lal (I. E. 715)	Madhya Pradesh	Nil	"	7.0	27.0	34.0
Madua (I. E. 707)	Bihar	1.5	"	4.0	28.0	33.5
Madua (I. E. 624)	Uttar Pradesh	1.0	"	3.0	27.0	31.0
Pyru Ragi (I. E. 22)	Andhra Pradesh	2.0	"	1.0	23.0	26.0
Madua (I. E. 703)	Bihar	Nil	"	Nil	22.0	22.0
Ragi (I. E. 464)	Mysore	Nil	"	Nil	21.0	21.0
Vellai Kanni (I.E. 344)	Madras	1.0	"	1.0	14.0	16.0
Masburi Mandia (I.E. 509)	Orissa	1.0	"	Nil	6.0	7.0
Kodah (I. E. 691)	Bihar	1.0	"	Nil	2.0	3.0
Agni Kuvaragu (I.E. 398)	Madras	Nil	"	Nil	2.0	2.0
Vellai Surattai (I. E. 381)	Madras	Nil	"	Nil	1.0	1.0

It is evident from Table 1 that Madua (I. E. 246, 137, and 707) from Bihar, Madia lal (I. E. 715) from Madhya Pradesh and Madua) (I. E. 624) from Uttar Pradesh, were heavily infected and showed loss of 48.5, 34, 33.5, 34 and 31 per cent, respectively, due to seed-borne fungi. While Vellai Surattai (I. E. 381) and Agni Kuvaragu (I. E. 398) from Madras, and Kodah (I. E. 691) from Bihar showed only slight infection, the loss being 1, 2 and 3 per cent, respectively.

Control with seed-dressing fungicides : Naturally infected seeds of variety Madua (I. E. 246) were divided into seven lots. Six of them were treated with fungicides at the rate of 2 gms./Kg. and the seventh

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

was left untreated to serve as control. 400 seeds of each treatment were plated on sterilized moist blotters to test the relative efficacy of the fungicide against seed-borne infection. The data obtained are summarized in Table 2.

TABLE 2 : Efficacy of different fungicides against seed-borne fungi of *ragi*

Fungicide	Active ingredient	Percentage infection of	
		Fusarium sp.	*H. nodulosum*
Agrosan GN	tolylmercury acetate	Nil	Nil
Ceresan	phenylmercury acetate	Nil	Nil
Flit 406	n-(trichloromethylmercapto)-4-cyclohexene-1,2-dicarboximide	1.5	6.5
Fermate	ferric dimethyldithiocarbamate	0.5	6.5
Semesan	2-chloro-4(hydroxymercuri) phenol	Nil	9.0
Fytolan	copper oxychloride	2.0	28.5
Control	———	2.5	45.5

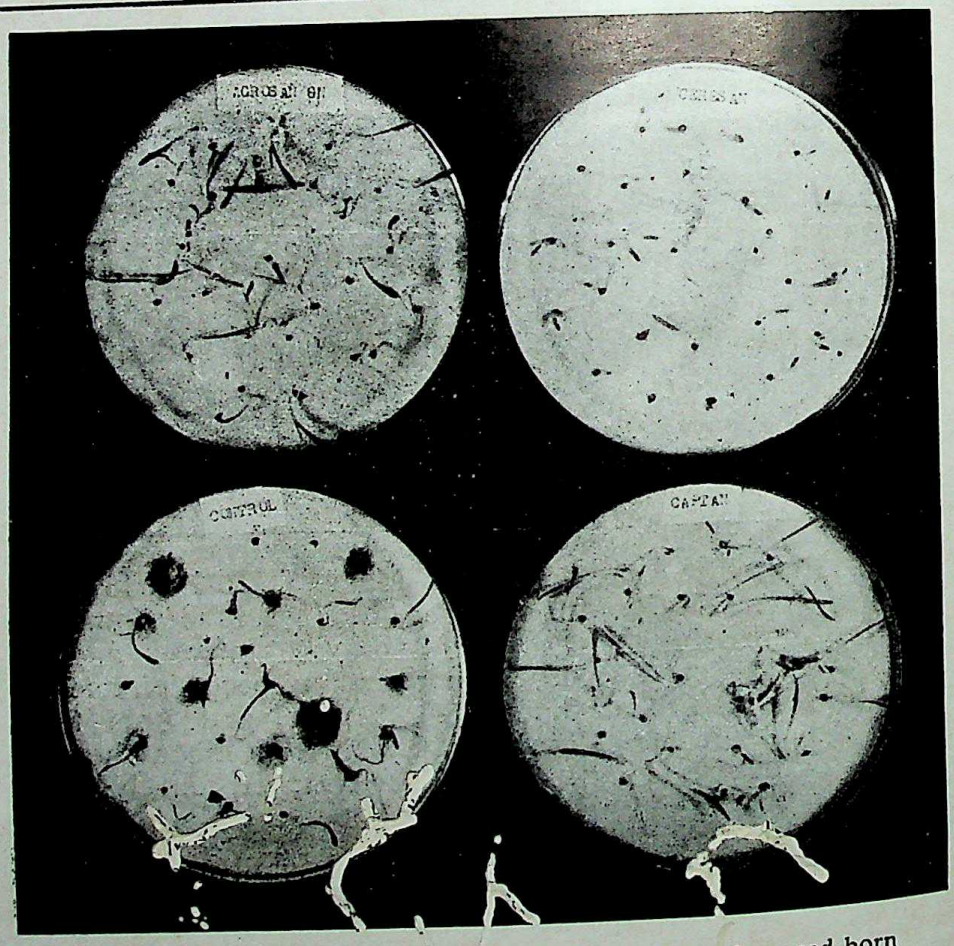


Fig. 2. Showing efficacy of different fungicides against seed-born fungi of *ragi*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The data in Table 2 show that Agrosan GN and Ceresan completely eliminated seed-borne infection of *Fusarium* sp. and *H. nodulosum* (Fig. 2). The untreated Control in the same tests gave infections as high as 2.5 and 45.5 per cent, respectively, of both these organisms. Flit 406, Fermate and Semesan reduced the infection to a great extent but could not eliminate it completely. Ceresan delayed germination by about 2 days whereas Agrosan GN treated seeds gave normal germination. Therefore, seed treatment with Agrosan GN is recommended for the control of seed-borne fungi of *ragi*.

SUMMARY

Tests conducted by Standard International Seed Testing Methods on thirteen varieties of *ragi* (*Eleusine coracana* Gaertn.) collected from seven different States of India showed the presence of *Helminthosporium nodulosum* Berk. and Curt., and species of *Fusarium*, *Aspergillus*, *Curvularia* and *Alternaria* on *ragi* seeds. Contamination of *H. nodulosum* was heavy on varieties: Madua (I. E. 246, 137 and 707) of Bihar, Madia lal (I. E. 715) of Madhya Pradesh and Madua (I. E. 624) of Uttar Pradesh, being 45.5, 34, 32, 34 and 30 per cent, respectively. Varieties Vellai Surattai (I. E. 381) and Agni Kuvaragu (I. E. 398) from Madras, and variety Kodah (I. E. 691) from Bihar, showed very little seed infection (*i. e.* 1 to 2 per cent). Infection of *Fusarium* sp. was 0-1.5 per cent in different varieties except in Pyru Ragi (I. E. 22) and Madua (I. E. 246) where it was 2 and 3 per cent respectively. Out of the six fungicides, namely Agrosan GN, Ceresan, Flit 406, Fermate, Semesan and Fytolan, tested against seed-borne fungi of *Ragi* Agrosan GN gave complete control of seed-borne pathogens without any adverse effect on germination.

ACKNOWLEDGEMENTS : Thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for encouragement and for providing necessary facilities of work and to Dr. D. Suryanarayana for going through the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

LEAF BLIGHT AND DRY STALK ROT OF STRAWBERRY CAUSED BY RHIZOCTONIA BATATICOLA

V. C. LELE AND H. C. PHATAK

(Accepted for publication November 30, 1964)

INTRODUCTION : Strawberry (*Fragaria* spp.) is a popular fruit used for making of ice creams and in canning industry. A highly perishable commodity, it is relished especially for its flavour. It is a crop of temperate regions and is usually grown in the hills in this country. Experiments are, however, under way to grow strawberries in the hotter plains. It is commonly known that large-fruited and productive varieties now in cultivation are derived from a chance crossing of but a few species of *Fragaria* and the crop is subject to many diseases.

A large number of strawberry plants of the experimental plots of the Division of Horticulture, Indian Agricultural Research Institute, New Delhi was found to be severely affected by a leaf-spotting and blighting disease in the month of September, 1963. Disease symptoms were also noticeable on other plant parts. On the leaves, more or less circular to spreading spots with ashy grey centre and purplish dark margin were conspicuous. The spots became oval to irregular in shape as the disease advanced (Fig. 1). The lesions appeared to be spreading from the margins of the leaf, towards its centre. In older plants puckering, leaf curling and defoliation appeared quite commonly. Runners and stalks of the affected plants turned dark brown or black with irregular lesions. New outgrowth from the affected plants was very

1

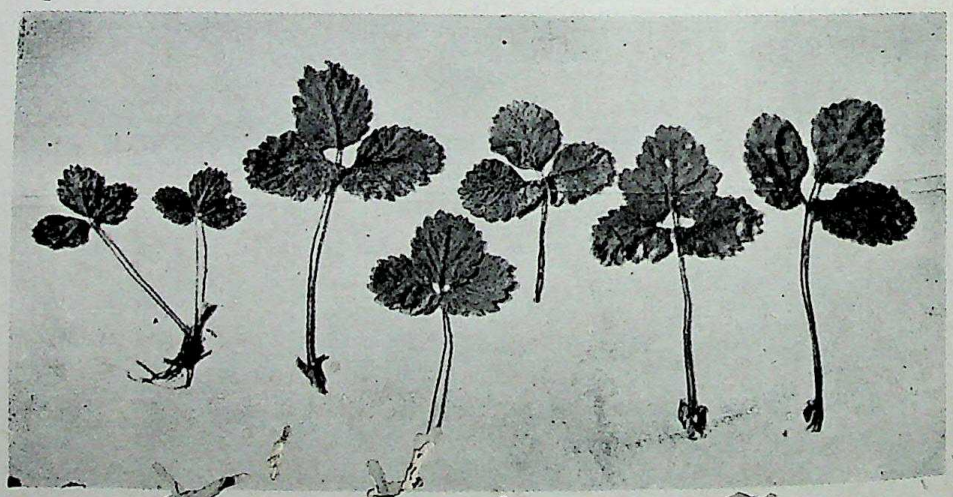


Fig. 1. Infected leaflets of strawberry (variety "Royal Sovereign"). Milder symptoms on leaflets towards the left side. Severe symptoms on the leaflets towards the right side. Note conspicuous leisions on the leaflet towards extreme right.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

much suppressed. The new roots were poorly developed and the older ones had a shredded appearance at their distal portions, but no regular rot was seen. Maximum damage was found to be done to "Royal Sovereign" (*Fragaria chiloensis* x *F. virginiana*), an exotic variety in which the mortality rate was as high as 90 per cent. The other two varieties growing by the side, namely, 'Pusa Early Dwarf' and 'Dilpasand' revealed a mortality rate between 60 and 80 per cent. Still others showed a moderate infection. No variety was found to be free from this disease.

EXPERIMENTAL : Microscopic examination of the affected plant parts revealed presence of fungal mycelium. No spores were, however, found in any sample, though this collection was pretty large. A few sclerotia were noticed on some of the runners and suckers. Isolations were made from the leaf spots, lesions from the runners, as well as, the roots on potato dextrose agar (P.D.A.) medium. Sub-culturing was done after 48 hours by transferring hyphal tips on to P.D.A., oatmeal *agar* medium and a medium in which sugarcane juice had replaced dextrose of the P. D. A. medium. Fungal growth appeared to be very similar in the isolates from various plant parts on each of the three media used in this study. The growth of the mycelium was profuse, fluffy and white in colour. Mycelium was broad, septate and branched. Within three days of sub-culturing small black, horny, fleshy sclerotia, variable in form were produced in abundance on the cultures. The sclerotia were formed among and connected by the mycelial threads. Test tubes and petri plates containing the culture were examined at regular intervals to see if the fungus had sporulated. No sporulation was, however, seen even after 20 weeks. Heating and desiccation of the cultures to various degrees also failed to induce sporulation.

PATHOGENICITY : For inoculation purposes, a few healthy plants of strawberry variety 'Dilpasand', raised in glasshouse for almost a year were employed. Young and old leaves, as also the petioles were inoculated with a loopful of hyphal mass having sclerotia from a culture on P.D.A. After inoculation, sterile distilled water was sprayed by means of an atomiser on the inoculated plants as also on the uninoculated plants acting as control. The plants were kept in a moist chamber for 72 hours to maintain high humidity. The temperature of the chamber was between 33° and 40°C. Lesions appeared on inoculated leaves and petioles within five days and presently, the leaves showed the characteristic spotting and subsequent curling and withering (Fig. 2) at the end of three weeks when the photograph was taken. The control plants did not exhibit any of these symptoms and remained absolutely normal. The fungus was readily re-isolated on P.D.A. from the inoculated plant parts showing infection.

DISCUSSION : Considering the various morphological and pathological features of the fungus described above, it becomes evident that it is *Rhizoctonia batatikola* (Taub.) Butl., the pycnidial stage [*Macrophomina phaseoli* (Maubl.) Ashby] of which was not found in nature, nor

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

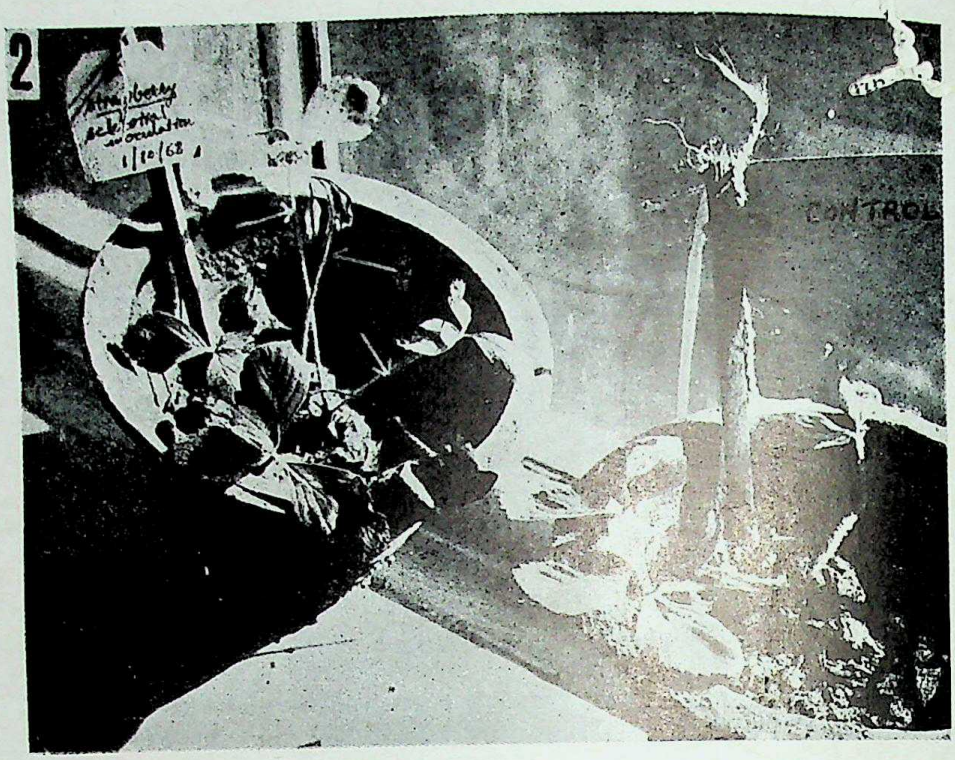


Fig. 2. Pathogenicity test; note curling of inoculated leaflets indicated by arrows. (Photographed 3 weeks after inoculation).

could it be produced in culture. It is apparent from the work of Haigh (1930) and Luttrell (1946) that the fungus is a highly variable one and that non-pycnidial strains are common. The present one appears to be a strain of this kind. The fungus is known to have a wide host range. The strawberry, however, appears to be a new host on which the fungus is recorded in this country. Also there appears to be, under natural incidence. a differential varietal response to this disease as observed in field. This aspect requires to be studied further.

Many diseases caused by *Rhizoctonia* species have been recorded from various countries. Dodge and Stevens (1924), Martin (1926) and Demaree (1948) have recorded pathogenic *Rhizoctonia* spp. on strawberries in the U. S. A. Diseases due to *Rhizoctonia solani* have been reported by Coons (1924) from the U. S. A. and by McDonald (1925) from Kenya, Africa. A disease caused by *R. bataticola* has been dealt with by Tompkins and Gardner (1935) in the U. S. A. They failed to produce pycnidial stage of the fungus by varying temperature. Teich and Ernstein (1961) from Israel and Johnson *et al.* (1962) from California, U. S. A. have reported *Macrophomina phaseoli* on strawberries. Obviously, they seem to have encountered the pycnidial stage of the fungus on this host. The disease symptoms described by us, however, seem to be different from those described by the above workers in as much as the main target of malady appeared to be the leaves and other

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

vegetative parts of the infected plants and the fungus, having been repeatedly and invariably associated with a 'Cortical Root Rot Complex' was found to be devoid of this feature. The damage to the fruits, none the less, was only indirect, that is, through the loss of foliage. This also was a distinguishing feature. This difference in the symtoms picture may be due to a difference in the strains or due to a change of environment. It is significant that newly or lately introduced varieties showed a higher rate of mortality attributed to this disease, than those being grown in the country for some time earlier. The fungus under consideration has not only a wider host range, but is known to be soil-borne and seed-borne. It is associated with crop sequence as also with soil and weather conditions that subject the crop to extensive heat or drought at a critical stage in its development (Andrus, 1938). It appears, therefore, that a temperate-region crop like strawberry was exposed to such extreme conditions more commonly in the plains (Delhi), where this pathogenic fungus built up quickly and developed unabatedly, notwithstanding how it established itself or was present already. In fact, such a situation did exist. A severe drought and high temperatures preceded disease outbreak and monsoon rains were much too delayed and scanty.

Strawberry is best grown vegetatively through suckers and runners and it is also not unlikely that the disease has been introduced, despite phyto-sanitary checks, along with planting material received from abroad on a large scale in the recent past. Given proper conditions, the build up from an initial low start may be rapid and devastating enough.

Recommendations were made to procure certified disease-free plants afresh and to set them in virgin fields or those kept fallow for quite some seasons, as also to avoid plantation in water-logged soil or one with improper drainage. Because of the adoption of some of these measures, the incidence of this disease on strawberry crop this year was much too less than seen in the past few seasons. A fair measure of success in combating the disease was being achieved.

SUMMARY

Rhizoctonia bataticola (Taub.) Butl. has been found to cause leaf blight and dry stalk rot of strawberry (*Fragaria* spp.) in the field at Indian Agricultural Research Institute, New Delhi.

Disease symptoms produced by the fungus appear to be different from those recorded by all other workers.

The fungus has been newly reported on strawberry in this country and appears to be highly pathogenic causing a large-scale destruction.

ACKNOWLEDGEMENT : Our grateful thanks are due to Dr. B. L. Chona for suggesting work on this problem and for his keen interest as also to Shri R. L. Manjal, for his useful suggestions and overall help throughout the period of these investigations. We are obliged to Dr. S. K. Mukherjee, Head of the Division of Horticulture, for his

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

cooperation and help in this work. Thanks are also due to Mr. H. K. Gorkha, photographer, for the photographs in the paper.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi

References

ANDRUS, C. F. (1938) Seed transmission of *Macrophomina phaseoli*. *Phytopathology*, **28** : 620—634.

COONS, G. H. (1924) Black root of strawberry. *Quart. Bull. Michigan agric. Coll.*, 7 : 25—26.

DEMAREE, J. B. (1948) Diseases of strawberries. *U. S. Dept. of Agric. Farm. Bull.* No. 1891.

DODGE, B. O. AND N. E. STEVENS (1924) The *Rhizoctonia* brown rot and other fruit rots of strawberries. *Journ. agric. Res.*, **28** : 643—648.

*HAIGH, J. C. (1930) *Macrophomina phaseoli* (Maubl.) Ashby and *Rhizoctonia bataticola* (Taub.) Butl. *Roy. Bot. Gard. Ann., Peradeniya, Ceylon.* **11** : 213 – 249.

*JOHNSON, H., A. H. HOLLAND, A. O. PAULUS AND S. WILHELM (1962) Soil fumigation found essential for maximum strawberry yields in southern California. *Calif. Agric.* **16** : 4—6.

LUTTRELL, E. S. (1946) A pycnidial strain of *Macrophomina phaseoli*. *Phytopathology*, **36** : 978—980.

*MARTIN, W. H. (1926) Report of the department of Plant Pathology. *Forty-sixth Ann. Rept. New Jersey Agric. Exper. Stat.* for the year ending June 30, 1925 : 443 – 457.

*MCDONALD, J. (1925) Annual Report of the mycologist for the year ended 31st December, 1924 : 106—111.

*TEICH, I. AND Z. ERNSTEIN (1961) Hadbarat mahalot u mazzikim be -Tutsadeh be 1961 (The control of diseases and pests of strawberry in 1961). *Hassadeh*, **41** : 673.

TOMPKINS, C. M. AND M. W. GARDNER (1935) Relation of temperature to infection of bean and cowpea seedlings by *Rhizoctonia bataticola*. *Hilgardia*, **9** : 219—230.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON LEAF BLIGHT OF INGA DULCIS

A. K. Roy

(Accepted for publication November 30, 1964)

During July 1963, a leaf blight was observed on the hedge plants of Madras thorn (*Inga dulcis* Willd.) around the campus of the Mycologist's Office, Borbheta, Assam. The disease started on the lamina as small, grey spots of more or less globose or oblong shape, measuring from 2—11 mm. in diameter. They appeared either inside the margin or at the edge proceeding inwards. Gradually the spots enlarged blightening half or more than half portion of the leaf resulting in their fall. No other part of the host was affected. The disease appeared in a very mild form. On the blighted portion minute, black, pinhead like dots appeared which on examination were found to be the acervuli of *Pestalotia albo-maculans* P. Henn. originally recorded on *Dalbergia* sp. from Rio de Janero, Brazil (Guba, 1961). In Assam, it was reported on *Flemmingia* sp. from Balahari, Kamrup (Mundkur and Kheswalla, 1942).

Acervuli sub-epidermal, erumpent mostly epiphyllous and rarely hypophyllous, 100-186 μ in diameter. Conidia clavate cylindric to elliptic fusoid, $16-20 \times 6-7\ \mu$, the two upper coloured cells are usually darker than the lower one, setulae 2—3, $13-28\mu$ long, pedicels upto 5.5μ long. On potato dextrose agar the growth is very thick, white, effuse and much aerial. Acervuli developed after 3 days at room temperature.

Material and Methods : Large scale isolations were made from leaves showing typical symptoms and as all of them yielded the same fungus, a representative type was selected. Monosporic isolation was done to maintain the pure culture. The stock was kept on potato dextrose agar slants with a pH of 6 at room temperature varying from 80 to 92° F. The fungus grows well both on P. D. A. and Richard's agar and therefore, for nutritional studies the latter was selected. The fungus was grown in petri dishes cc. using 15 dishes of medium and after the development of the mycelial mat, discs of 10 mm. diameter were cut by sterile cork borer and were inoculated at the centre of the petri plates. Growth and sporulation was recorded after 7 days on solid medium in petri dishes kept at room temperature. For each of the treatment three replications were taken Measurement of sporulation was done in four categories : very good, good, moderate and scanty based on comparative number of acervuli.

Pathogenicity Test : A few healthy twigs with fully grown leaves were selected and detached from the plant. After surface sterilization of the leaf surfaces light scratches were made with sterile needle, conidial suspension smeared and kept in a beaker of water covering with

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

a bell jar. Under these conditions acervuli appeared after three days. It was found that the waxy coating present on the leaf surface needed to be removed for infection by the fungus. When scratches were not made and only waxy coating removed by rubbing with sterile cotton swab before inoculation acervuli appeared after 4-5 days.

Similar test was made on the leaves of *Dalbergia sissoo* Roxb. on which the acervuli developed within the same period as on *Inga dulcis*. Afterwards, cross inoculation was tried on the latter host with the culture from the former which also proved successful suggesting that the same organism was causing disease on both.

PHYSIOLOGICAL STUDIES : *Growth and pH* : Seven ranges of pH from 4 to 10 were selected to see their effect on growth and sporulation of the pathogen. The observations are recorded in Table 1. It is observed that the fungus grows at a wide range of pH, best in 4 to 5, closely followed by 6 to 7. The growth gradually decreases after 7 and sharply falls at 10. pH on the other hand has very little effect on sporulation as zonation appears in all the cases.

TABLE 1 : Growth and sporulation of *P. albo-maculans* on different pH ranges.

pH	Av. diam. of colony (mm.)	Sporulation	Cultural characters
4	*	Good	Colony white, effuse, thick, fluffy. Sporulation starts after 3 days in a zonate fashion.
5	*	Very good	Growth same as 4. No. of acervuli maximum.
6	93	Moderate	Growth more or less same as in the above case, much aerial mycelium, minute acervuli develop after 3 days throughout the mat.
7	93	Moderate	Same as above but thin growth.
8	88	Moderate	Growth thin, acervuli very small and develop along the zones.
9	77	Moderate	Growth scanty, sporulation same as above.
10	47	Moderate	Growth very sparse but sporulation is not affected.

*Petri dishes (95 mm. diam.) completely filled up.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Utilization of carbon : The fungus was grown on Richard's medium from which sucrose was substituted by equal quantity of carbon of other compounds. Medium without carbon source did not support any growth.

It is seen from Table 2 that glucose supports the maximum growth, followed by maltose, starch, sucrose, lactose. Growth is poor in glycerol and mannitol. In glycerol mycelial mat is not produced. In all the disaccharides and glucose sporulation is good, maximum being in sucrose. Starch supports medium sporulation, mannitol scanty and glycerol nil. Zonation appears in all cases except in glycerol.

TABLE 2 : Growth and sporulation of *P. albo-maculans* on different carbon sources.

Carbon source	Av. diam. of colony (mm.)	Sporulation	Cultural characters
Glucose	*	Good	Growth very thick, white, fluffy. Acervuli first develop along the zones and then spread throughout the colony, 3 ciliate conidia more than the 2 ciliate ones.
Lactose	80	Good	Growth thinner, less aerial mycelium, a great number of acervuli are submerged in the medium.
Sucrose	81	Very good	Mycelial mat medium thick.
Maltose	92	Good	Growth very thick just like glucose, size of acervuli is somewhat bigger than the other cases. 2 ciliate conidia are more than the 3 ciliate ones.
Starch	84	Moderate	Growth just like in sucrose, less fluffy.
Glycerol	69	Nil	Growth is very sparse, no mat is formed.
Mannitol	64	Poor	Mycelial mat of medium thickness, growth is almost like that of sucrose and starch but thinner than glucose and maltose.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Utilization of Nitrogen : In this case also potassium nitr[illegible] substituted by equal quantity of nitrogen of other compounds. [illegible]ys. It without nitrogen source supported little growth. [illegible]to be [illegible]

TABLE 3 : Growth and sporulation of *P. albo-maculans* on different nitrogen sources

Nitrogen source	Av. diam. of colony (mm)	Sporulation	Cultural characters
Potassium nitrate	81	Very good	Colony effuse, white, thick, fluffy, saltant of triangular shape and greyish.
Ammonium nitrate	84	Moderate	Growth thick as in the above case, many submerged acervuli develop, 3 ciliate spores are more than 2 ciliate ones.
Ammonium titrate	78	Good	Growth thicker than the above cases, 3 ciliate conidia are more than the 2 ciliate ones as in the above case.
Ammonium chloride	34	Nil	Colony punctiform with slight rosy tinge, aerial mycelium is very scanty.
Sodium nitrite	Nil	Nil	
L-asparagine	86	Moderate	Growth very thick as in case of ammonium titrate, profuse aerial mycelium, 3 ciliate conidia more than the 2 ciliate ones.
Dl-serine	68	Nil	Mycelial mat thin in all the cases and hardly seen, aerial mycelium almost nil.
Glutamic acid	45	Poor	Growth very thick, much fluffy with slight rosy tinge, many submerged acervuli develop, most of the spores 3 ciliate, 2 ciliate spores hardly [illegible]en.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Table 3 shows that best growth was obtained in L-asparagine followed by ammonium nitrate, potassium nitrate, ammonium titrate. Though in Dl-serine moderate growth was observed on the basis of colony diameter mycelial mat produced was very thin and sparse. On the other hand, in glutamic acid though the average diameter was not good, mycelial mat was very thick and fluffy. Except in Dl-serine, ammonium chloride and glutamic acid, zonation appeared in other cases. Saltant appeared on potassium nitrate. Sporulation was best on potassium nitrate, followed by ammonium titrate. L-asparagine and ammonium nitrate supported moderate sporulation which was scanty in glutamic acid and nil in Dl-serine.

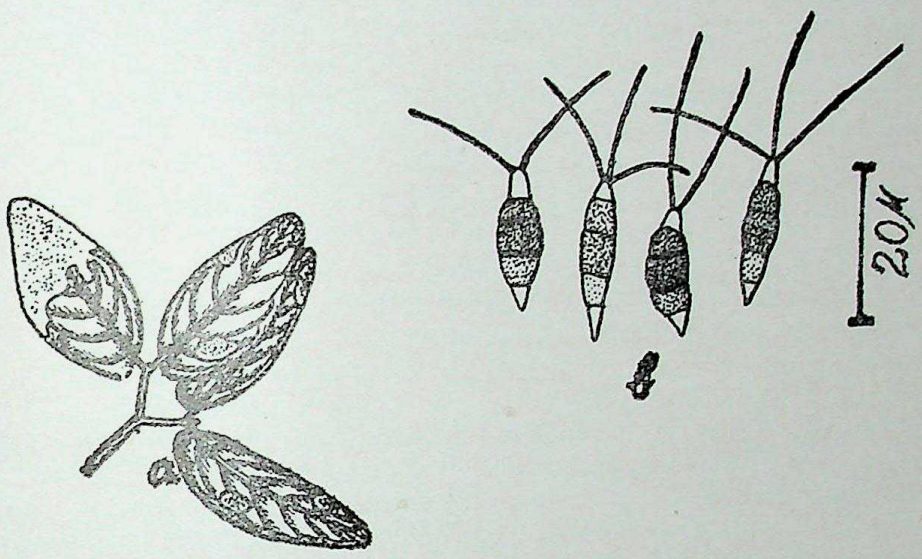


Fig. a. Infection (natural size); b. Conidia

DISCUSSION : As the disease could manifest easily when light wounds are made on the lamina or when the waxy coating was removed, *P. albo-maculans* appeared to be a weak parasite.

The pH studies showed that the pathogen is acid loving as regards growth and is in accord with the work of Patel. Kamat and Hingorani (1950) and Sarkar (1960-a and 1960-c).

Utilization of carbon sources by fungi depended upon the structural configuration of the compounds as well as on the capacity of the particular organism (Lilly and Barnett, 1951). As the general rule (Hawker, 1947) glucose was found to be utilized most readily by the fungus. Among all the disaccharides that support good growth, maltose gave the best result which was in accord with the performance of *Venturia inaequelis* (Cke.) Wint (Lafuze, 1937). Very good growth in starch agrees with the performance of *Gloeosporium psidii* Delacr. (Thind and Sandhu, 1956) which is probably due to the presence of starch splitting enzyme. Sugar alcohols are generally utilized with some difficulty as is evident by very poor growth in mannitol. Very scanty growth and no sporulation in glycerol is in accord with *Pestalotia psidii* Pat. (Patel *et al.*, 1950). *Gloeosporium muscum* Cooke and Massee, *G. papayae* P. Henn., *Colletotrichum papayae* (P. Henn) P. Henn. (Grewal. 1957) and *Cercospora viticola* (Ces.) Sacc. (Sethi and Munjal. 1963)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Little growth of the fungus in a medium devoid of nitrogen may be due to the presence of traces of nitrogen carried with the inoculum, which probably supports the growth at the beginning to some extent (Chandwani and Munjal, 1963; Sethi and Munjal, 1963). The test fungus utilizes an organic source, asparagine best as a nitrogen source which agrees with the performance of *Helminthosporium gramineum* Rab. (Chandwani and Munjal, 1963) and *Pestalotia mangiferae* Butl. (Sarkar, 1960-a). Next best growth occurs in nitrate nitrogen which has been shown by various workers (Patel *et al.*, 1950; Sarkar, 1960-b; Thind *et al.*, 1956). Good growth in ammonium nitrate may be due to the presence of nitrate ion. No growth in nitrite nitrogen is a common phenomenon in fungi. The toxic effect of nitrite can be explained that it is unstable in acid solution and nitrous acid destroys proteins and amino acids (Lilly *et al.*, 1951). Best sporulation in potassium nitrate is in accord with the performance of *Pestalotia psidii* Pat. (Patel *et al.*, 1950) *P mangiferae* Butl. (Sarkar, 1960a) and *Colletotrichum hibisci* Poll. (Sarkar, 1960 b).

Summary

A leaf blight of *Inga dulcis* caused by *Pestalotia albomaculans* was observed at Borbheta, Assam. Pathogenicity tests on it and *Dalbergia sissoo* were successful. The pathogen grows at a wide range of pH from 4 to 10 ; best being in 4 to 7 decreasing at higher levels. pH had little effect on sporulation which was same from 6 to 10. Glucose supported the maximum growth of the fungus, followed by starch and disaccharides. Glycerol and mannitol gave poor result. Sporulation was good on disaccharides and glucose, poor in mannitol and nil in glycerol. L-asparagine supported the best growth as nitrogen source, followed by ammonium nitrate, potassium nitrate and ammonium titrate. In ammonium chloride and Dl-serine growth was poor and sporulation nil. No growth occured in nitrite nitrogen. Sporulation was good in nitrate nitrogen and poor in glutamic acid.

Acknowledgement : Thanks are due to the staff of Mycology Section, particularly to Mr. P. C. Gohain and Shri M. K. Shome, for their help during the course of the studies.

Division of Mycology,
Department of Agriculture, Assam,
Jorhat.

References

Chandwani, G. H. and R. L. Munjal (1963) Studies on physiology of *Helminthosporium gramineum* Rab., the causal agent of stripe disease of barley. *Indian Phytopath.*, **16** : 301—306.

Converse, R. H. (1953) The influence of nitrogenous compounds on the growth of *Helminthosporium gramineum* in culture. *Mycologia*, **45** : 335—344.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

GREWAL, J. S. (1957) Effect of carbon nutrition on growth and sporulation of some anthracnose fungi. *Indian Phytopath.*, **10** : 121—126.

GUBA, E. F. (1961) *Monograph on Monochaetia and Pestalotia* : 207

*HAWKER, L. E. (1947) Further experiments on growth and fruiting of *Melanospora destruens* shear, in the presence of various carbohydrates with special reference to the effects of glucose and of sucrose. *Ann. Bot.* (n. s.) **11** : 245-259.

LEBEN, CURT AND G. W. KEITT, (1948) *Venturia inaequalis* (Cke.) Wint. V. The influence of carbon and nitrogen sources and vitamins on growth *in vitro*. *Amer. J. Bot.*, **35** : 337—343.

LILLY, V. G. AND H. L. BARNETT (1951) *Physiology of fungi.* McGraw Hill Book Co. Inc. New York.

LAL, A. AND K. V. GEORGE (1953) Blight of *Eryobotrya japonica* caused by *Pestalotia longi-aristata*. *J. Phytopath. Soc.*, B. H. U., **2**.

MUNDKUR, B. B. AND K. F. KHESWALLA (1942) Indian and Burman species of the genus *Pestalotia* and *Monochaetia*. *Mycologia*, **34** : 308—317.

PATEL, M. K., M. N. KAMAT AND G. M. HINGORANI (1950) *Pestalotia psidii* Pat. on guava. *Indian Phytopath.*, **3** : 165—175.

SARKAR, A. (1960a) Leaf spot disease of *Mangifera indica* L. caused by *Pestalotia mangiferae* Butl. *Lloydia*, **23** : 1—7.

SARKAR, A. (1960b) Studies on Anthracnose fungus of *Hibiscus cannabinus* L. *Lloydia*, **23** : 97—101.

SARKAR, A. (1960 c) Comparative studies of two races of *Colletotrichum falcatum* Went. *Sci. and Cult.*, **26** : 83.

SETHI, K. K. AND R. L. MUNJAL (1963) Studies on nutritional requirements of *Cercospora viticola* (Ces.) Sacc. (*Mycosphaerella personata* Higgins). *Indian Phytopath.*, **16** : 185—194.

THIND, K. S. AND R. S. SANDHU (1956) Studies on the nutrition of *Gloeosporium psidii*, incitant of guava anthraconse. *Indian Phytopath.*, **6** : 207—208.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CULTIVATION OF SOME PLANT VIRUSES IN CALLUS TISSUE CULTURES

S. P. RAYCHAUDHURI AND M. D. MISHRA

(Accepted for publication November 30, 1964)

A number of viruses have been established in cultivated roots, tumour and callus tissue cultures. White (1934) was the first who successfully cultured roots of tomato plants infected with tomato aucuba mosaic virus. Subsequently, Morel (1948) cultivated callus tissue from galls incited by *Agrobacterium tumefaciens* (Sm. and Towns.) Conn. on tobacco plants infected with tobacco mosaic virus (TMV) and potato viruses X (PVX) and Y (PVY). These cultures remained infective when cultured serially for an indefinite period. Nickell (1955) and Black (1957) obtained considerable information with regard to the growth of the cultured tissue derived from tumours of plants infected with the wound tumour virus. However, the concentration of the virus in the cultured tissues was not estimated.

The present investigation was undertaken with a view to establishing a type culture collection of commonly occurring plant viruses in Delhi.

MATERIAL AND METHODS : Experiments were conducted with the normal callus tissues obtained from the stem cambium of the virus affected tobacco plants. Four viruses, namely, chilli mosaic (ChMV) (Mishra, 1963), sunn-hemp mosaic (SMV) (Raychaudhuri, Nariani and Das, 1962), a ringspot strain of PVX (Sharma and Raychaudhuri, 1962) and the type strain of TMV, were cultivated on normal callus cultures.

The technique for culturing the tissue and preparing the medium was the same as described earlier (Raychaudhuri and Mishra, 1962). After a growth period of one to two months the callus tissues were cut into equal portions for serial transfers. Three or four such portions of the cultured tissues were retained for estimating the virus contents. These pieces were washed two to three times in vials containing 0.5 per cent Na_2SO_3 in order to remove agar remnants. Excess of moisture was removed by gently blotting them. After weighing, these pieces were ground with a few drops of 0.5 per cent Na_2SO_3; later, distilled water equal in quantity per gram weight of the tissue was added. The standard extracts thus prepared were utilised for assaying the virusc ontents by inoculating the local lesion hosts with a spatula, the flat end of which was wrapped with muslin. *Chenopodium amaranticolor* L. *Cyamopsis tetragonoloba* (L.) Taub., *Gomphrena globosa* L. (Wilkinson and Blodgett, 1948) and *Nicotiana glutinosa* L. (Holmes 1928) were used as

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

local lesion test plants for ChMV, SMV, PVX and TMV, respectively. Serial transfers were made at regular intervals and virus contents estimated for a period of two years. Each experiment was repeated three times and their mean values were taken into account.

The tubes containing tissue cultures were kept in an air-conditioned room with a temperature ranging from 20° to 27°C. and lighted with fluorescent lights.

RESULTS : *Cultivation of plant viruses in tissue culture* : At regular intervals, the callus growths containing different viruses such as ChMV, SMV, PVX and TMV, were subcultured on the basal medium. Virus contents of the cultured tissues were periodically estimated for two years by local lesion technique. Only actively growing portions of the cultures were employed for quantitative estimation. The results are presented in Table 1.

TABLE 1 : Virus contents in the callus growths at different intervals

Viruses	Virus contents at different durations (in months) (Average number of local lesions per four leaves)					
	2	4	8	12	18	24
ChMV	45	40	41	45	42	40
SMV	89	82	80	84	82	80
PVX	18	11	15	17	14	12
TMV	62	62	60	61	59	60

The virus contents in the callus cultures were found to remain almost constant during the period. However, only 25 per cent of the transfers remained actively growing by the end of two years. In the case of ChMV, longevity of which in the expressed sap is very short, even the non-dividing sectors of the callus growths showed the presence of the virus after two years.

Concentration of the viruses in different tissues : The virus contents in the leaf lamina and stem pith were compared with that in the callus growths, grown on the synthetic medium. The origin of these tissues was from tobacco plants of the same age, height and vigour and inoculated with the respective viruses. The average concentration of the viruses in these tissues were as given in the Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Concentration of viruses in different tissues of tobacco plants and the callus growth

Tissues	Average number of local lesions per three leaves			
	ChMV	SMV	PVX	TMV
Leaf tissue	162	189	25	197
Stem pith	52	50	10	70
Callus tissue	47	48	8	65

The virus content of leaf lamina was found to be more than four times the concentration of the virus present in the tissue culture in case of ChMV and SMV. However, in case of all the four viruses tested, the virus content of the stem pith was about the same as that of the callus growth.

DISCUSSION : Augier de Montgremier and Morel (1948) estimated the concentration of TMV in tissue culture extracts to be 1/30 to 1/40 of the amount present in the sap from leaves of the plant grown in soil. In our studies the virus concentration in the cultured callus tissue was found to be almost the same as in the stem pith of the plants grown in soil. This is due to the fact that concentration of the virus in leaves remains higher than that in the pith of infected plant, which is generally taken as the source of the cultured tissue. Segretain (1943), however, found the inoculated newly formed callus tissue to contain as much virus as in infected leaves.

After repeated transfers, the virus content in the actively growing callus tissues cultivated on the basal medium was found to remain constant for a period of two years. It may, therefore, be presumed that the virus in the actively growing cells may be multiplying *in vivo* along with the cells of the cultured tissues. Augier de Montgremier and Morel (1948) considered that the concentration of the virus is affected by the rapidity of tissue proliferation. Kassanis (1957), however, could not find any correlation between growth of the tissue and virus concentration. In our studies, we have also detected the presence of virus in sufficient concentration even in the non-dividing callus cells.

SUMMARY

Four viruses, namely, chilli mosaic, sunn-hemp mosaic, tobacco mosaic and potato virus X, were successfully cultivated and maintained in normal callus tissue cultures. Their concentrations in the cultureb tissue remained almost the same for a period of two years in repeated transfers.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The virus contents of the leaves were found to be about three to four times that of the concentration in the callus growth, which was almost the same as that of the stem pith.

ACKNOWLEDGEMENT : The authors are much indebted to Dr. B. P. Pal. Director, for the kind encouragement received in developing this work and also to Dr. B.L. Chona, Head of the Division of Mycology and Plant Pathology, for his interest in the project.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

AUGIER DE MONTGREMIER, H. AND G. MOREL (1948) Sur la diminution de la teneur en virus (*Marmor tabaci* Holmes) de tissues de Tabac cultives *in vitro*. *Compt. rend.*, **227** : 688-689.

BLACK, L. M. (1957) Viruses and other pathogenic agents in plant tissues cultures. *J. Nat. Cancer Inst.*, **19** : 663-685.

HOLMES. F. O. (1928) Accuracy in quantitative work with tobacco mosaic virus. *Bot. Gaz.*, **86** : 66-81.

KASSANIS, B. (1957) The multiplication of tobacco mosaic virus in culture of tumorous tobacco tissues. *Virology*, **4** : 5-13.

MISHRA, M. D. (1963) Host range and physical properties of a virus causing mosaic symptoms and necrosis on chillies (*Capsicum annuum* L.). *Indian J. Microbiol.*, **3** : 79-84.

MOREL, G. (1948) Recherches sur la culture associee de parasites obligatories et de tissus vegétaux. *Ann. Epiphyt.* (*n.* s.), **14** : 1-112.

NICKELL, L. G. (1955) Nutrition of pathological tissues caused by plant viruses. *Amee biol.*, **31** : 107-121.

RAYCHAUDHURI, S. P. AND M.D. MISHRA (1962) Cultivation of a mosaic virus isolated from chilli in tissue culture. *Indian Phytopath.*, **15** : 185-187.

RAYCHAUDHURI, S. P., T. K. NARIANI AND C. R. DAS (1962) *Cyamopsis tetragonoloba* (L.) Taub.—a local lesion host of sunn-hemp mosaic virus. *Indian Phytopath.*, **15** : 79-80.

SEGERTAIN, (1943) Culture d'un virus et son inoculation sur fragments de tige de tabac cultives *in vitro*. *Ann. Inst. Pasteur.*, **69** : 61-63.

SHARMA, D. C. AND S. P. RAYCHAUDHURI (1962) Occurrence of a ring spot strain of potato virus X. *Indian Phytopath.*, **15** : 250-253.

WHITE, P. R. (1934) Multiplication of the viruses of tobacco and aucuba mosaics in growing excised tomato root tips. *Phytopathology*, **24** : 1003-1011.

WILKINSON, R. E. AND E. M. BLODGETT (1948) *Gomphrena globosa*, a useful plant for qualitative and quantitative work with potato virus X (Abstr.) *Phytopathology*, **38** : 28.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE GENUS PRINGSHEIMIA IN INDIA

S. T. TILAK AND RAMCHANDRA RAO

(Accepted for publication November 30, 1964)

The genus *Pringsheimia* was originally described by Schulzer[3] with *Pringsheimia rosarum* as the type. However, it remained unrepresented in the Indian flora for a long time. Recently Müller[2] and Kapoor and Gill[1] have described a new species each. The genus is characterised by the partly or fully sunken ostiolate ascocarps. Several aparaphysate, bitunicate asci originate from the basal convex-cushion or short columner cushion. The ascospores are uniseriate or biseriate, hyaline with transverse and vertical septa.

During their investigations of different genera of ascomycetes from India, the authors noticed some black fungus, heavily infecting dried stems of *Ailanthus excelsa* Roxb. and *Acacia melanoxylon* Br. A careful study revealed that these belong to the genus *Pringsheimia*. Since no species have been reported on these hosts so far, detailed comparative studies were carried out. It was found that the two collections on hitherto unreported hosts differ in several morphological features.

1. *Pringsheimia acaciae* Tilak and Ramchandra Rao. sp. nov.

Fructificationes globosae vel ovoideae, superficiales vel patim immersae in textus plantae hospitis, $115-210 \times 84-105\ \mu$. Parietes brunnei vel nigri, efformati cellulis parite crasso praeditis, $12-18\ \mu$. Ascocarpus ad maturitatem aperitatem aperitur irregulariter ad apicem superenum. Asci plures originem ducentes ex serie basali, parietibus tenuibus ornati, bitunicati, $50-70 \times 7-11.5\ \mu$. uniseriatae, hyalinae, primo transversis tantum, postea septis quoque verticalibus septatae, $18-22 \times 8-10\ \mu$.

Fruit bodies globose to ovoid, superficial or partly embedded in host tissue, $115-210 \times 84-105\ \mu$, wall brown to dark $12-18\ \mu$. Outermost cells thick-walled polygonal, inner cells thin-walled, elongated hyaline to subhyaline. Ascocarps open irregularly at maturity. Asci numerous, clavate to cylindrical, stipitate, bitunicate, thin-walled, $50-70 \times 7-11.5\ \mu$. Ascospores 8, hyaline, 3—5 septate, septa horizontal but later a vertical septum originates in each cell, $18-22 \times 8-10\ \mu$.

On dry stems of *Acacia melanoxylon* Br. from Ooty, during months of Nov. 1963, Leg. Kadge, T. S. (Herb. of Marathwada University, no. MUH 130).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

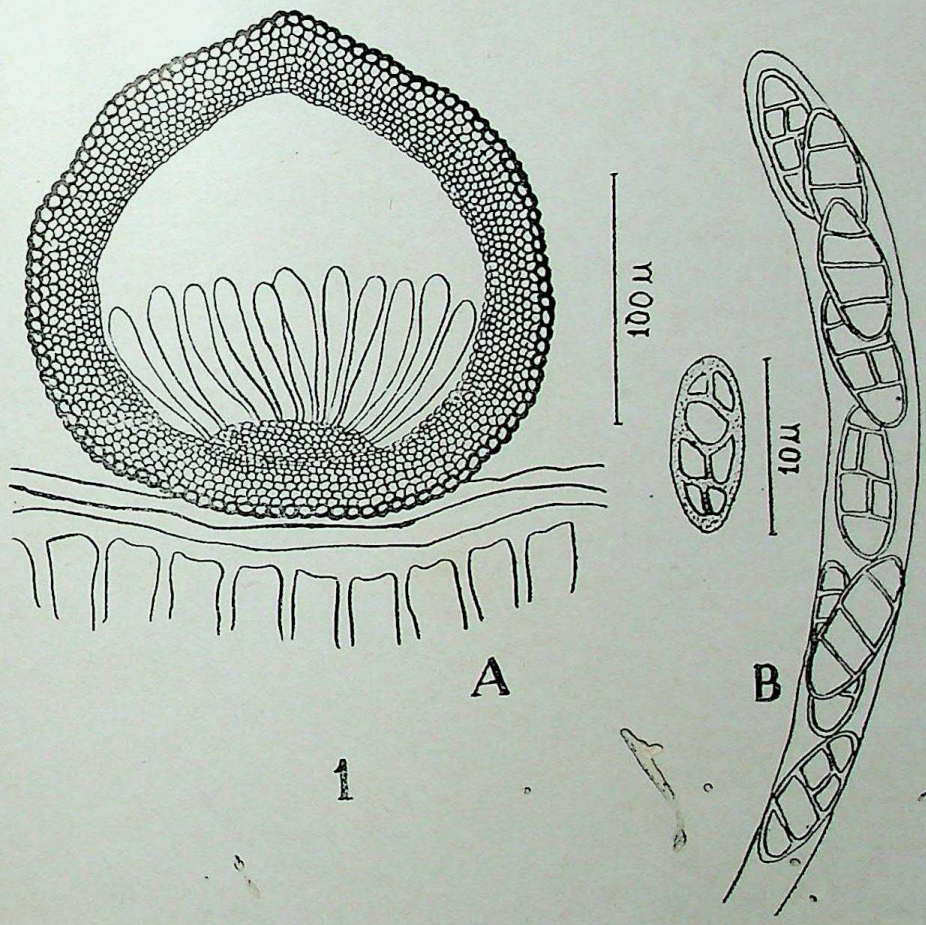
Digitized by Arya Samaj Foundation Chennai and eGangotri

2. *Pringsheimia ailanthi* Tilak and Ramchandra Rao sp. nov.

Fructificationes globosae vel ovoideae, superficiales vel partim in textus plantae hospitis immersae, 97—140×52—96 μ. Cellulae exteriores crassis parietibus praeditae, interiores verotenuibus. Ascocarp us irregulariter aperitur ad maturitatem. Asci plures, clavati vel cylindrici bitunicati, parietibus praediti, 26—54×6—16 μ. Ascosporae 8, biseriatae hyalinae, 19—38×11—19 μ, 3—5 septatae, septis tum horizontalibus tum verticalibus.

Fruiting bodies globose to ovoid, superficial or partly embedded, 97—140×52—96 μ. Outermost cells thick-walled, inner cells thin-walled. Ascocarp opens irregularly at maturity. Asci numerous, cylindrical, bitunicate, thin-walled, 26—54×6—16 μ. Ascospores 8, hyaline, irregularly biseriate, 19—38×11—19 μ, transverse septa 3—5, vertical septa also present.

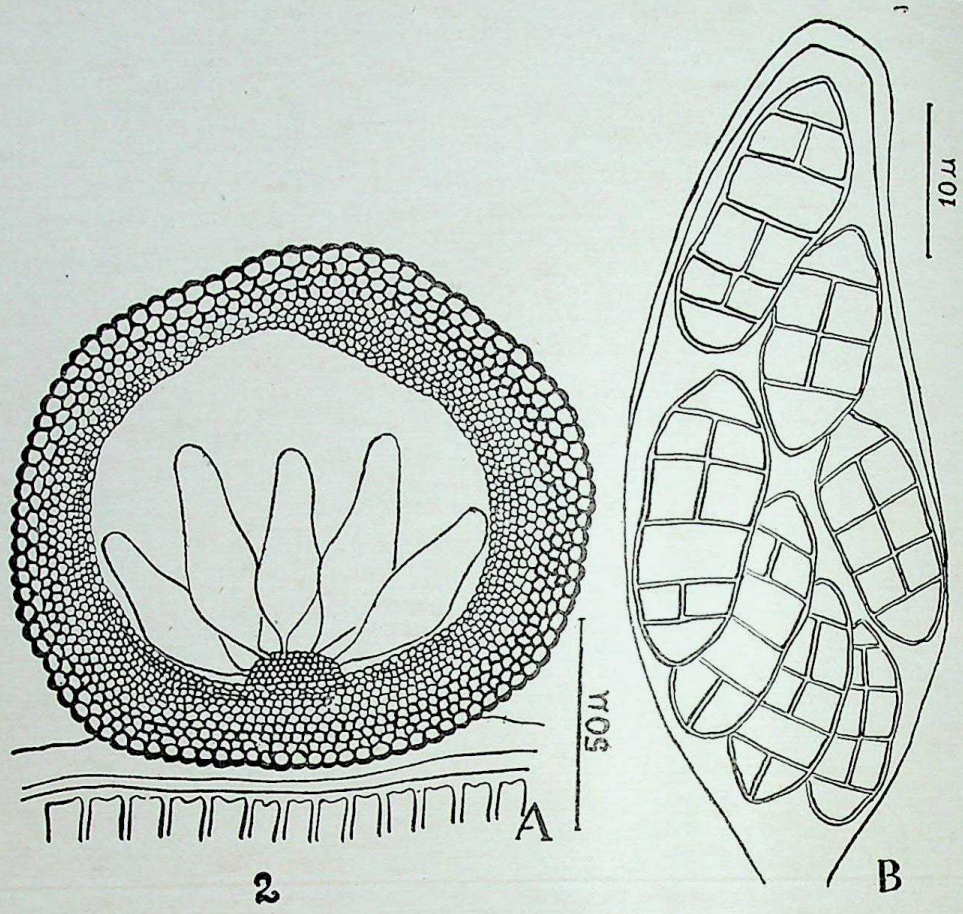
On dry stems of *Ailanthus excelsa* Roxb., from Aurangabad, during months of Feb-March, 1964, Leg. Ramchandra Rao (Herb. of Marathwada university, no. MUH. 126.)



1

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



2

3. *Pringsheimia cynodontis* Kapoor and Gill. Ind. Phytopath., **14**: 149-153 (1961).

On dry leaves of *Cynodon dactylon* Pers. from Delhi., Ascospores irregularly biseriate. This is the only Indian species collected on leaves.

4. *Pringsheimia sepincola* (Fr.). Höhnel-Ann. Mycol., **18** : 97 (1920)

Müller recently reported this species from India on stems of *Rubus ellipticus* Sm. at Naini Tal (Ratighat).

Spores are upto 4-6 celled. The species is typical in having 4-6 celled ascospores.

5. *Pringsheimia smilaicis* Müller.-Sydowia. **11** : 454-472 (1957)

The fungus has been collected by Emil Müller from Kuman, Naini Tal, on dried stems of *Smilax parvifolia* Wall.

Comparsion between five species of Pringsheimia from India

Indian spp	Ascocarp	Ascus	Ascospores
P. acaciae	115—210 × 84—105μ	50—70 × 7—12μ	18—22 × 8—10 μ.
P. ailanthi	97—140 × 52— 96μ	26—54 × 6—16μ	19—38 × 11—19μ
P. cynodontis	50—80 μ (diam.)	60—70 × 28—32μ	28—32 × 10—12μ.
P. sepincola	60—90 × 40—60μ	24—42 × 15—22μ	15—23 × 7—11μ.
P. smilaicis	100—180 × 90—120μ	40—45 × 14—16μ	17—20 × 5.5—6.5μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Key to Indian species of Pringsheimia

a. Perithecia on leaves—*P. cynodontis.*

b. Perithecia on stems, ascospores biseriate—

bb. ascospores irregularly biseriate— *P. ailanthi.*

bcd. ascospsores 3—5 septate, vertical septa 2—3. *P. sepincola.*

bce. ascospores only transverse septate— *P. smilaicis.*

c. Ascospores uniseriate— *P. acaciae.*

Müller[2] has recently reviewed and critically discussed the taxonomic position of the genus.

The genus *Pringsheimia* is an addition to the generic list of Maharashtra State.

The type materials have been deposited in the Herbaria of Marathwada University, Aurangabad and Cryptogamie Indiae Orientalis, New Delhi.

Summary

The present paper deals with the Indian species of the genus *Pringsheimia*. The fungi have been studied from type material, fresh collections and herbarium specimens. In all five Indian species viz., (1) *P. acaciae* sp. nov., (2) *P. ailanthi* sp. nov. (3) *P. cynodontis* Kapoor and Gill., (4) *P. sepincola* (Fr.) Höhnel and (5) *P. smilacis* Müller have been studied. A key has been provided for identification of these species.

Acknowledgements : The authors are thankful to Dr. Emil Müller, Swiss Federal Institute of Technology, Zürich, and to Dr. B. L. Chona, Head of the Division of Mycology, Indian Agricultural Research Institute, Delhi, for the supply of some type material and to Rev. Father Dr. H. Santapau, Chief Botanist, Botanical Survey of India, Calcutta, for the Latin diagnosis of new species.

Department of Botany,
Marathwada University, Aurangabad,
Maharashtra State, India.

References

1. Kapoor, J. N. and H. S. Gill (1961) *Indian Phytopath.*, **14** : 149-153.
2. Müller, E. (1957) *Sydowia*, **11** : 454472.
3. Schulzer (1886) Verh. z.-b. Ges. Wien, **16** : 57.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON CAPE GOOSEBERRY MOSAIC DISEASE

S. P. CAPOOR AND D. C. SHARMA*

(Accepted for publication December 4, 1964)

A mosaic disease of cape gooseberry, *Physalis peruviana* L., was observed in commercial plantations in several localities near Poona and the Agricultural College Farm over a period of several years. Affected plants were much dwarfed and had reduced and slightly curled leaves showing severe puckering with dark green blisters (Fig. 1). The older

Fig. 1. Field infected cape gooseberry plant showing characteristic symptoms of the mosaic disease.

*Present address : Resources and Scientific Research Division, Planning Commission, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

leaves on diseased plants usually shed prematurely leaving the twigs almost naked. Same was true of fruits, of which a high percentage dropped off while still green and immature.

Inoculation made initially with undiluted juice of infected cape gooseberry leaves to *Nicotiana tabacum* L. var. White Burley, *Datura inoxia* Mill. and *Nicotiana glutinosa* L., induced development of numerous bright yellow and round spots with a speck of necrosis in the centre on tobacco leaves (Fig. 2). The leaves of *D. inoxia* were curled downwards and showed mild vein clearing followed by mosaic mottling,

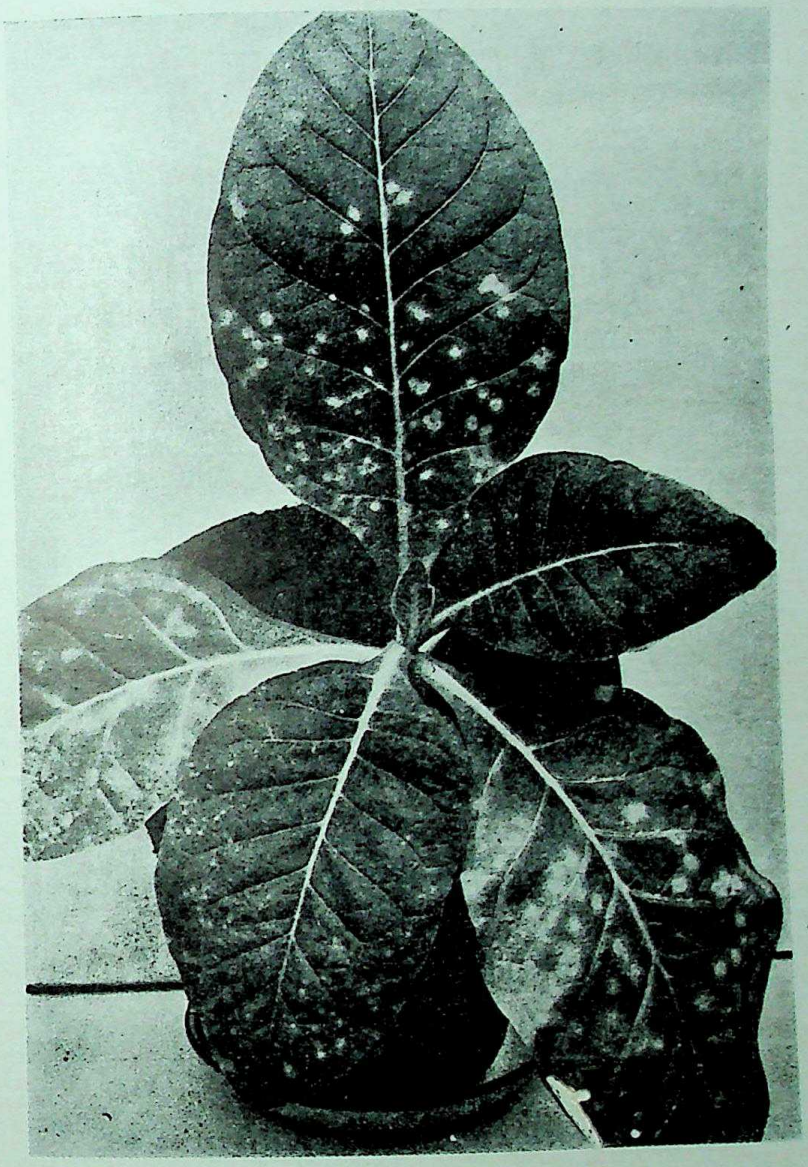


Fig. 2. *Nicotiana tabacum* var. White Burley, showing primary as well as systemic development of bright yellow spots on leaves following infection by the cape gooseberry mosaic virus.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

while small necrotic lesions appeared in leaves of *Nicotiana glutinosa*. These lesions were 1 – 1.5 mm. in diameter and brownish in colour. In this respect they appeared to be different to those produced in this host by the type strain of tobacco mosaic virus, although the virus appeared to be a strain of tobacco mosaic virus.

MATERIALS AND METHODS : All experimental plants were raised from seed and the seedlings transplanted in 4-inch earthen pots in well manured river soil. Inoculations were made by the leaf rubbing method using freshly expressed and diluted sap of leaves of infected cape gooseberry plants with the help of 600-mesh carborundum powder. The inoculated leaves were rinsed with tap water immediately after the inoculation. Cape gooseberry was used both as source plant and as test plant in most experiments.

Insects used in transmission trials were *Aphis gossypii* Glover collected from cotton, *Aphis rumicis* Linn. collected from *Solanum nigrum* L., *Myzus persicae* (Sulz.) collected from *Brassica oleracea* L. and *Macrosiphum pisi* Kalt. collected from *Pisum sativum* L. A species of grasshopper (unidentified), collected from cape gooseberry and cabbage plants, was also used in transmission trials of the virus. In all tests the insects were killed by spraying with a 0.02 per cent solution of Folidol after they had fed on test plants.

EXPERIMENTAL RESULTS : *Transmission of the Virus* : The virus was readily transmitted by mechanical inoculation of the infective juice and also by grafting cape gooseberry seedlings.

Tests by Insects : The aphid species, *Myzus persicae*, *Aphis rumicis*, *A. gossypii* and *Macrosiphum pisi*, collected from their respective natural host plants were starved for 1 to 2 hours before feeding them for 5 to 60 minutes on virus source, and again allowed post-acquisition fasting of at least 60 minutes before liberating them on test plants. In limited tests none of the aphid species could transmit the mosaic virus.

Grasshoppers were starved for 6 hours and then fed individually for 5 minutes on infected cape gooseberry on which they did feed only after starvation. They were then liberated individually for 18 hours on test plants to feed at will. In this test 13 plants were exposed to grasshopper feeding but none of these were infected.

By Seed : Seed was collected from infected cape gooseberry plants and sown in flats in the following season inside the glasshouse. In all 576 seedlings were obtained but all were healthy. The virus, therefore, does not appear to be carried in seed of infected cape gooseberry.

Through Soil : In October, 1956, fifty cape gooseberry plants were transplanted in a plot in the field, and when established were experimentally infected by inoculating each with the virus. In March, 1957, all the diseased plants were ploughed down in the soil and in the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

following rainy season (July, 1958) 50 healthy cape gooseberry seedlings were transplanted in the same plot after a light manurial dressing. These plants continued to grow healthy throughout the season. In March 1959, a random sample from these plants was indexed for the virus on tobacco but in no case any virus was recovered from them.

In another experiment cape gooseberry seedlings were grown in wooden trays inside the glasshouse. When about 12 cm. high they were inoculated with the virus and when fully infected were cut back to soil level leaving the stumps in the soil and allowed to dry. Fresh seed of cape gooseberry was then sown in the same soil. All the cape gooseberry seedlings remained healthy throughout the growth period. These tests showed that the virus is not soil-borne.

Through Picking : It is believed that the virus spreads from diseased plants to healthy ones through the hands of field workers and cultivators contaminated during cultural operations and harvesting of ripe fruits. The possibility of field contamination through picking was demonstrated in an experiment where 55 healthy cape gooseberry seedlings were contaminated through plucking one or two lower leaves from each of them with contaminated hands. Only 9 plants from these first showed the disease symptoms, but gradually all were infected within 28 days. It is, therefore, clear that much of the damage to the crop is done by the workers themselves through negligence and ignorance.

Host Range and Symptoms : Out of the plants tested, the virus infected the plants belonging to the family Solanaceae only and also *Gamphrena globosa* L. No infection was obtained in *Cucumis sativus* L., *Luffa cylindrica* Roem., *L. acutangula* Roxb., *Phaseolus vulgaris* L. var. Local, Pinto and Early Golden Cluster, *P. lunatus* L., *P. limensis* L., *Vigna sinensis* Savi., *Clitoria ternatea* L., *Cyamopsis tetragonoloba* (L.) Taub. *Desmodium gangeticum* DC., *Dolichos biflorus* L., *Crotalaria juncea* L., *Raphanus sativus* L., *Impatiens balsamina* L., *Ipomoea muricata* L., *Chenopodium album* L., *Zinnia elegans* Jacq., *Helianthus annuus* L., *Aster* sp., *Vinca rosea* L., *Abelmoschus esculentus* Moench., and *Gossypium arborcum* L.

Nicotiana tabacum—White Burley Tobacco. Originally when the virus was first collected it induced local bright yellow spots followed by a similar systemic infection in tobacco (Fig. 2) and later development of severe mosaic. Since the virus culture has been maintained in cape gooseberry through repeated transfer from plant to plant by juice inoculation, it seems to have reverted to a form which now induces vein clearing of young leaves of tobacco following inoculation and severe mosaic mottling later. Plants are very much dwarfed due to retardation of growth.

Nicotiana sylvestris. Local necrotic spots appear on the inoculated leaves, and occasionally systemic mild mosaic.

Nicotiana tabacum × *N. glutinosa*. F_1 hybrid tobacco.—Only local necrotic lesions appeared on the inoculated leaves. There was no systemic development of mosaic in this plant.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Physalis minima. Local necrotic spots appeared 4 days after inocuiation followed by leaf abscision. No systemic infection occurred probably because the leaves were shed before virus could spread within the host.

Nicotiana affinis, N. grandiflora, Physalis floridana, Capsicum frutescens, Petunia hybrida, Solanum nigrum and *S. nodiflorum.* All these developed mild or severe mosaic mottling, but local necrotic lesions did not appear.

Lycopersicon esculentum (tomato). Occasionally few necrotic spots appeared on inoculated leaves but the characteristic disease in tomato was a severe mosaic mottling.

Gomphrena globosa. Globe Amaranth. Crimson red necrotic lesions appeared on inoculated leaves.

Physical Properties :

Resistance to heat. In order to determine the resistance to heat and thermal inactivation of the virus, 3 ml. juice expressed from systemically infected cape gooseberry plants was exposed to various temperatures for 10 minutes in thin walled test tubes. Tubes were then cooled in ice cold water immediately after heating and the infectivity of the virus was tested by inoculating the treated juice on to *Nicotiana glutinosa.* Juice heated at 94° C for 10 minutes remained infective, but the virus was completely inactivated by exposure to heat at 96° C.

Tolerance to dilution : Freshly expressed juice diluted to successive 10-fold dilutions and inoculated to *N. glutinosa* gave positive infection at a dilution of 1 in 10^6, but not at 1 in 10^7.

Longevity in vitro : Juice expressed from diseased cape gooseberry leaves diluted 1 : 1 with water was stored in glass stoppered vials at room temperature (20 to 32° C). Aliquots tested for infectivity at intervals on cape gooseberry seedlings showed that the virus was still infective after storage for two years.

Immunological relationships : *Nicotiana sylvestris* leaves inoculated with Johnson's tobacco virus 1 three days before super-inoculation with CGMV did not develop necrotic lesions, while characteristic necrotic lesions appeared on leaves of plants inoculated with CGMV alone. This protection against infection with CGMV confirmed that the virus is a strain of TMV.

Origin and Spread of Infection : Field observations at Poona showed that the disease usually appeared late in season somewhere in December or January. Available information led to the view that the chief source of infection in the new crop is the farm labourer and his implements. On account of unhygienic conditions of cultivation and ignorance about virus diseases, the cultivator introduces TMV strains

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

from other crops, viz., potato, tomato, pepper and egg plant, during cultural operations.

Control Measures Suggested : The control of cape gooseberry mosaic virus would depend much on preventive measures or minimizing the initial field infection, and clearing the pockets of infection foci. The following precautionary measures are therefore suggested :

1. Diseased cape gooseberry plants should be rogued out and destroyed.

2. Each individual bush should be so trailed on a pole as not to come in contact with the neighbouring ones in order to avoid transmission through contact.

3. Workers should avoid successive contact of diseased and healthy plants in carrying out cultural practices in the field. They should wash their hands and implements thoroughly before working in a gooseberry field.

DISCUSSION : Cape gooseberry mosaic virus (CGMV) reported herein, resembles to some extent the tomato aucuba mosaic strain of TMV in the production of local necrotic lesions in *N. sylvestris*. At higher temperatures prevailing during the summer months in Poona a mild mosaic occasionally appears, otherwise the virus does not easily cause a systemic disease in *N. sylvestris*. In this respect and also in not infecting bean the CGMV resembles a tomato mosaic strain of TMV studied by Melchers *et al.* (1940) and the rib-grass strain of TMV reported by Holmes (1941). It also deserves consideration as a distinctive strain of TMV, its identity with this virus being clearly shown by the characteristic symptoms produced in *N. tabacum* and *N. glutinosa*, by its resistance to heat (remained infective after 10 minute exposure at 94° C), and its inability to cause necrotic lesions in *N. sylvestris* previously infected by the type strain of TMV.

There is no previous record of TMV or any of its strains causing disease in cape gooseberry in India although it is grown extensively for its attractive fruits in the southern, central and northern India, and also in the hills in the south and in the north. Strains of TMV, however, are known to infect cape gooseberry and other species of *Physalis* in nature in other parts of the globe. Allard (1914) was the first to report infection of tobacco mosaic virus in *Physalis* sp. Later, Crawford (1921) observed that *Physalis longiflora* acted as a symptomless carrier of TMV. *Physalis alkekengi* also was found to be a carrier of TMV by Nishimura (1918) in Japan, while Blattny (1959) reported that *P. alkekengi* acted as a systematic host of a green strain of TMV if the inoculation was repeated 3-4 times a day for 5-6 days. Stelzner in 1943 observed spontaneous infection of *P. alkekengi* in nature by a special strain of TMV. Tomato mosaic virus, which is also a strain of TMV (Smith, 1957), is known to affect *P. subglabrata*, *P. virginiana* and *P. heterophylla* is nature (Gardner and Kendrick, 1923). Under experimental conditions TMV could infect *P. peruviana* (Chamberlain, 1937), a masked strain of TMV infected *P. angulata* (Holmes, 1934), and tomato spotted wilt virus infected *P. peruviana* (Bald and Samuel, 1931).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In our tests the CGMV was not transmitted by any of the aphid species used in transmission trials or by the unidentified species of grasshopper. Although transmission of TMV in nature has not been adequately proved, Hoggan (1931, 1934) showed that TMV was transmitted by *Macrosiphum euphorbiae* and *Myzus persicae*. Olitsky (1925) and Newton (1953) observed that *Pseudococcus citri* (Risso) transmitted TMV in tobacco and in *Nicotiana glutinosa*. Walters (1951, 1952) demonstrated TMV transmission from tobacco to tobacco by the grasshopper *Melanoplus differentialis* (Thomas). Suguira and Hirayama (1958) found that TMV was transmitted in 15 per cent of test tobacco plants by the caterpillars of *Barathra brassicae* L. Chant (1959) obtained transmission of the Cowpea strain of TMV in Cowpea and *Mucuna aterrima* (Holl.) by the Galerucid beetle (*Ootheca mutabilis* Sahlb.).

Since CGMV is not aphid-borne nor does it infect *Cucumis sativus* L., it is more than established that it does not belong to the group of cucumber mosaic viruses.

Summary

A mosaic disease of cape gooseberry, *Physalis peruviana* L., observed over a period of several years in commercial plantations in Poona was found to be caused by a virus which initially produced numerous bright yellow and round spots with a speck of necrosis on leaves of *Nicotiana tabacum* var. White Burley followed in it by a similar systemic disease and later into a severe mosaic mottle. The cape gooseberry mosaic virus (CGMV) is not transmitted by *Myzus persicae* (Sulz.), *Aphis rumicis* Linn., *A. gossypii* Glover, *Macrosiphum pisi* Kalt., or by an unidentified grasshopper. It is also not transmitted through seed or soil, but is transmitted to a very high percentage through plucking of leaves from infected and healthy plants simultaneously. The virus deserves consideration as a distinctive strain of TMV because it produces characteristic symptoms in *N. tabacum* and *N. glutinosa*, it does not infect cucumber, it has a remarkable resistance to heat and it did not produce lesions on *N. sylvestris* previously immunized by type strain of TMV. Possible measures for the control of the disease have been indicated.

Indian Agricultural Research Institute,
Plant-Virus Research Laboratory,
Poona-5.

References

Allard, H. A. (1914) The mosaic disease of tobacco. *U. S. Dept. Agri. Bull.*, **40**.

Bald, J. C. and G. Samuel (1931) Investigations on spotted wilt of tomatoes. II. *Australian Counc. Sci. and Ind. Res. Bull.*, **54**.

*Blattny, C. (1959) *Physalis alkekengi* L. Jako prfznakovy hostitel Zeleneho kmene TMV Typ. *Preslia Praha*, **31** : 182—184.

*Papers not seen in original.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHAMBERLAIN, E. E. (1937) Tobacco mosaic—Its appearance, cause and control. *New Zealand J. Agric.*, **4** : 163—174.

CHANT, S. R. (1959) Viruses of Cowpea, *Vigna unguiculata* L. (Walp.), in Nigeria. *Ann. Appl. Biol.*, **47** : 565—572.

CRAWFORD, R. F. (1921) Overwintering of mosaic on species of *Physalis*. *Phytopathology*, **11** : 47.

GARDNER, M. W. AND J. B. KENDRICK (1923) Field control of tomato mosaic. *Phytopathology*, **13** : 372—375.

HOGGAN, I. A. (1931) Further studies on aphid transmission of plant viruses. *Phytopathology*, **21** : 199—212.

HOGGAN, I. A. (1934) Transmissibility by aphids of the tobacco mosaic virus from different hosts. *J. agric. Res.*, **49** : 1135—1142.

HOLMES, F. O. (1934) A masked strain of tobacco mosaic virus. *Phytopathology*, **24** : 845—873.

HOLMES, F. O. (1941) A distinctive strain of tobacco mosaic from Plantago. *Phytopathology*, **31** : 1089—1098.

*MELCHERS, G., G. SCHRAMM, H. TRURNIT, AND H. FRIENDIRICH-FREKSA (1940) Die biologische, chemische und elektronenmikroskopische Untersuchung eines Mosaik Virus ous Tomaten. *Biol. Zentralb.*, **60** : 524—556.

NEWTON, W. (1953) Transmission of tobacco mosaic by citrus mealybug. *F. A. O. Plant Prot. Bull.*, **2** : 40.

NISHIMURA, M. (1918) A carrier of mosaic disease. *Bull. Torry. Bot. Club.*, **45** : 219—233.

OLITSKY, P. K. (1925) The transfer of tobacco and tomato mosaic disease by the *Pseudococcus citri*. *Science*, **62** : 442.

SMITH, K. M. (1957) A text book of Plant Virus Diseases. (Second Edition). J. and A. Churchill Ltd. London.

*STELZNER, G. (1943) Spontaneous infection of *Physalis alkenkengi* by a special strain of tobacco mosaic. *Angew. Bot.*, **25** : 259—268.

*SUGIURA, M. AND S. HIRAYAMA (1958) Transmission of tobacco mosaic virus by the larvae of *Barathra (Mamestra) brassicae* L. Mie Daigaku Nogakubu Gakujutsu Hokoku **16** : 47—63.

WALTERS, H. J. (1951) Grasshopper transmission of three plant viruses. *Science*, **113** : 36.

WALTERS, H. J. (1952) Some relationships of three plant viruses to the differential grasshopper, *Melanoplus differentialis* (Thos.). *Phytopathology*, **42** : 355—362.

*Papers not seen in original

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RUSTS CONNECTED WITH BARBERRY IN CENTRAL NEPAL

D. P. MISRA

(Accepted for publication December 9, 1964)

Large number of rusts have been reported to occur on or are known to be connected with Barberry in various countries of the world. From India, there have been reports of *Aecidium berberidis* determined as *Puccinia graminis* var. *agropyri* by Prasada (1947); *A. montanum* (by Butler 1905); *P. brachypodii* Ramakrishnans (1949), and *P. poae-nemoralis* (Joshi and Payak, 1963). However, the out-break of *P. graminis* var. *tritici* through Barberry has not been found to occur in nature, in this country. It appears that there have been no such reports from Nepal, so far. Therefore, to study the role of various species of *Berberis* in relation to different rusts, areas of Central Nepal were surveyed from Butwal (400 m. a. s. l.) in the south to Muktinath (3, 600 m. a. s. l.) in the north during March-May of 1961. These areas constitute climatically and topographically different regions varying from sub-tropical to temperate zones, with annual rainfall ranging from about 500 cms. in the south to 25 cms. in the northern dry zone, where there are heavy snows during winters. Because of streams and rivulets and high hills that flank them, different species of *Berberis* and grasses are found well scattered in comparatively a small area. Such conditions are also responsible for adoption of crop practices so as to suit local conditions. To give an instance for this, wheat is cultivated as winter crop in most of the region, though the time of harvesting ranges from April to July from place to place, and in area like that of Muktinath it is cultivated during summers so as to be harvested during October-November.

During the survey pycnial and aecial stages on many species of *Berberis* were found in the hills between the altitudes of 1,500 and 3,600 m. a. s. l. In close proximity of such infections, fresh, as well as overwintered uredial and telial stages of rusts on *Agropyron* and *Brachypodium* were found. Near Tukuche (3,000 m. a. s. l.) and at Muktinath overwintered straw of wheat bearing uredia and telia was collected near fresh pycnial infections on Barberry during early May. Similar wheat straw was common in the fields in this area. However, the standing summer crop of wheat was free from rusts. The available material of rusts, plants and seeds of grasses, wheat and species of *Berberis* and *Mahonia* from various places was brought to the Simla laboratory of the Indian Agricultural Research Institute, where morphological and pathological studies were conducted. These results are presented in this paper.

EXPERIMENTS AND RESULTS : *1. Rust on Brachypodium connected with barberry* :—Covered telia on *B. sylvaticum* that had overwintered in nature in Northern Nepal (2-3,200 m. a. s. l.) were found to germinate

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

readily. These were inoculated on young leaves of plants raised from seeds and root-wood (collected from Nepal) of the species of *Berberis* and *Mahonia*. Pycnia followed by aecia developed on *B. lycium*, *B. aristata* and *B. mucrifolia* in 10-15 days at average range of temperatures 10-18°C., whereas, no infection appeared on *B. insignis* and *M. acanthifolia* under these conditions. Aeciospores from aecia thus formed, produced uredia on *Brachypodium sylvaticum* but failed to infect *Agropyron* and local wheat collected from Nepal.

Morphological characters of all the stages of the rust thus developed in the laboratory and uredia and telia collected on *B. sylvaticum* from Tukuche, Jhumse, Lete, Marpha, Ghara and Muktinath as well as aecia collected on *B. aristata* and *B. mucrifolia* from most of the aforesaid places, were found to agree with the description of *P. brachypodii* (Joerstad, 1951 and Payak, 1965).

2. *Rust on Agropyron connected with barberry* :—Naked telia on *Agropyron* sps. were collected from the hills (between 2-3,000 m. a. s. l.). The teliospores which readily germinated in the laboratory, were found to produce pycnia and aecia on *B. lycium*, was earlier reported as *B. koehneana* and *B. aristata* within 10-15 days after inoculation *B. insignis* and *M. acanthifolia* remained immune in these tests. Fresh aeciospores from these infections produced uredia followed by telia in a few days on *Agropyron* sps., but failed to infect *B. sylvaticum* and wheat.

Morphological characters of the stages of this rust produced in the laboratory and telia found on *Agropyron* sps. as well as aecia collected on *B. koehneana* from Ghorepani, Dhampu, Jhumse and Tukuche were found to be similar to *P. g.* var. *agropyri* described by Prasada (1947).

3. *Black rust of wheat and connected studies with barberry* :—Overwintered straw of wheat bearing uredia and telia of *Puccinia graminis* var. *tritici* were commonly found near Tukuche and at Muktinath, where wheat is a summer crop. The teliospores germinated readily and infected young leaves of *B. aristata*, *B. lycium*, *B. koehneana*, and *B. mucrifolia* whereas, *B. insignis* and *M. acanthifolia* were found to be immune. From the aeciospores of these infections, wheat, *Agropyron* sps. and *B. sylvaticum* were found to be susceptible and uredia developed in 12-15 days after inoculation.

In addition to this, twenty single aecial cup cultures were established on wheat and were tested for the physiologic races involved. The reactions on differential varieties (Stakman *et al.*, 1962) with these cultures are presented in the table.

None of the races, given in the table have been picked up from the uredial field collections from India and Nepal. However, number 'IX' is identical to race 116, which was obtained as a segregate of race 21 through selfing on Barberry (Misra, 1962). Race 21 is common in India and Nepal. Number 'III' and 'VI' are similar to race 18 and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

214 respectively and other six races are not given in the key by Stakman *et al.*, (1962), are apparently new.

TABLE 1 : Reactions of 20 single aecial cup cultures from species of *Berberis* inoculated with overwintered teliospores of black rust of wheat from Nepal.

Differential Varieties	Race Category Number								
	I	II	III	IV	V	VI	VII	VIII	IX
Little club	4	4	4	4	4	4	4	4	4
Marquis	4	4	4	4	4	4	4	4	4
Reliance	4	4	4	4	4	4	0;	0;	0;
Kota	4	4	4	4	4	4	4	4	4
Arnautka	4	0;	4	4	4	0;	4	0;	4
Mindum	4	0;	4	4	4	0;	4	0;	4
Spelmar	X—3	3	0;—1	0;—1	4	0;	4	3—4	4
Kubanka	0;—1	0;—1	0;—1	0:—1	0;—1	0;—1	0;—1	X—3—	3—
Acme	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1	X—3—	3—
Einkorn	3	0;—1	0;—1	3	0;—1	4	0:—1	0;—1	0;—1
Vernal	0;—1	0;—1	0;—1	0;—1	3	0;—1	3	4	3
Khapli	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1	0;—1
No. of cultures	12	1	1	1	2	1	1	2	1
Reactions identical to races	—	—	187		—	214	—	—	116

Note : Two races were picked up from each of two cultures and from others only one race in each culture was found. Symbols designating reactions of races are after Stakman *et al.*, (1962)

At the time of survey during early May at Muktinath, aecia had not developed on Barberry and only pycnial stage was developing. At Tukuche, a bush of *B. koehneana* on which overwintered wheat straw bearing telia of black rust was found, aecia had developed on a few leaves. Morphologically it was not possible to distinguish this field collection with certainty as *P. g. tritici* against other collections of *P. g.* var. *agropyri* from field. The aeciospores had lost their viability by time these were brought to the laboratory and therefore, no pathological tests could be made.

4. *Aecial stage on barberry with unknown perfect stage* : Leaves and young twigs of *B. aristata*, *B. angulosa*, *B. mucrifolia* and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

B. koehneana at Ghasa, Jhumse to Tukuche, Kalopani, Ghorepani, Marpha and Devithan were found with profuse aecial infection. The infected host parts were atrophied and gave the appearance of Wich's-broom. The infection appeared to be systemic. No grass bearing uredia or telia was found in the proximity of such infections. Fresh aeciospores did not produces infection on transplanted *Agropyron* and *Brachypodium* and wheat on inoculation. Morphological characters of aecial material correspond to *A. montanum*.

CONCLUSIONS : Genetic relationship of *P. brachypodii* with Barberry was first demonstrated by Mayor (1933) from Switzerland. From Nepal this appears to be the first report for the rust to complete its life-cycle through the alternate host and *B. mucrifolia* has been found to be a new host in addition to *B. lycium* and *B. aristata* which get infected in India (Payak, 1965).

A. montanum has been found to occur on new hosts i.e. *B. angulosa*, *B. koehneana* and *B. mucrifolia* in Nepal in addition to the already known host *B. aristata* from India (Butler, 1905).

P. g. var. *agropyri* has been reported only from India (Prasada, 1947). The present report of the rust in Nepal, appears to be new. Besides, *B. koehneana* is found to be a new host for the rust on which the life-cycle is completed in nature, in addition to the known hosts, *B. lycium* and *B. aristata*.

In the areas of Muktinath, wheat straw, bearing germinable teliospores of *P. g.* var. *tritici* from the previous crop, was commonly found during the months of April-May. At this time new leaves on susceptible species of Barberry had also started appearing and wheat crop of the current summer season was in 2-4 leaf stage. With the expected temperature and humidity conditions to follow in this area. it is felt that there are strong chances for the rust to complete its life-cycle through the alternate host. Therefore, comprehensive surveys are necessary to show the extent of Barberry infection in such areas. Uredial collection on the summer crop of wheat may also throw light on the race flora of this region and these races can be compared with the reactions obtained by aecial cup cultures developed from the telia collected from Northern Nepal.

B. insignis and *M. acanthifolia* have been found to be immune to at least three rusts (*Puccinia*) reported in this paper.

ACKNOWLEDGEMENT : The author is greatful to Dr. R.S. Vasudeva, former Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, for his keen interest and useful suggestions during these studies. Thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, for his kind encouragement in this work and to Drs. R. Prasada and M. M. Payak for their valuable suggestions in the manuscript.

Indian Agricultural Research Institute,
Plant Pathological Research Sub-Station,
Flowerdale, SIMLA-2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

BUTLER, E. J. (1905) Some Indian forest fungi. *Indian Forester*, **31** : pp. 548.

JOERSTAD, I. (1951) The graminicolous rust fungi of Norway. *Sty. norshe Vidensk Acad. I.* **3** : 92 pp.

JOSHI, L. M. AND M. M. PAYAK (1963) A *Berberis Accidium* in Lahaul Valley, Western Himalyas. *Mycologia*, **55** : 247-250.

MAYOR, E. (1933) Notes Mycologiques VIII-*Bull. Soc. Neuchatel Sci. Nat.*, **58** : 7-31

MISRA, D. P. (1962) Selfing studies with races 21 and 42 of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. *Indian Phytopath.*, 15 : 203-6.

PAYAK, M. M. (1965) *Berberis* as the Aecial Host of *Puccinia branchypodii* in Simla Hills (India). *Phytopath. Z.*, **52** : 49-54.

PRASADA, R. (1947) Discovery of uredo-stage connected with the aecidia so commonly found on species of *Berberis* in the Simla Hills. *Indian Journ. Agric. Sci.* **17** : 137-151.

RAMAKRISHNAN, T. S. AND K. RAMAKRISHNAN (1949) Additions to Fungi of Madras. V. *Proc. Indian Acad. Sci.*, Sec. B. **28** : 56-70.

STAKMAN, E. C., D. M. STEWART AND W. G. LOEGERING (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici*. U. S. D. A. *Agric. Res. Ser.* E. 617 : 55 pp.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SOME NEW RECORDS OF THE ORDER MUCORALES FROM INDIA

A. K. SARBHOY

(Accepted for publication December 9, 1964)

INTRODUCTION : During the studies on soil and coprophilous fungi the author isolated about fifty species of Mucorales, out of which nine are new records from Indian soils.

MATERIALS AND METHODS : The method of taking soil samples was the same as used by Saksena and Mehrotra (1952). Soil samples were taken from first inch of soil to six inches. They were brought to the laboratory in sterilized containers. Isolation of Mucorales was done by Waksman's Direct Isolation (Waksman, 1916), Dilution method and also by Soil Plate methods (Warcup, 1950). Pure cultures of the isolates were made by picking few spores from a single fruiting head.

Each of the isolate was studied in detail on at least three media (Potato dextrose agar, Synthetic *mucor* agra and Hay extract agar).

Identification were made with the help of standard monographic treatments.

So far about 50 species of the order Mucorales have been isolated from nearly two hundred soil samples. A scrutiny of available literature showed that nine isolates were new records from Indian soils except *Mucor rouxianus*. These have been described here in details.

1. *Actinomucor elegans* (Eidam) Ben. and Hess, Fig. I—A to E.

Colonies rapidly growing on SMA attaining a diameter of 2 cm. in about 5 days ; at first white later turning tilleal buff (Ridg., Pl. XL 15) ; odour slightly acrid ; reverse colourless. Mycelium stoloniferous, stolons branched. Sporangiophores arising from the substrate mycelium, from aerial hyphae, from stolons opposite to the rhizoids, upto 35 μ in diameter, terminating in a large sporangium, with a whorl of three to six branches arising below the tip, each branch terminating into a secondary sporangium ; with cross walls typically located above the origin of branches or whorls of branches. Sporangia spherical, buff coloured in reflected light, drak gray in transmittent light ; terminal sporangia with deliquescent or persistent, smooth or spiny walls, usually 62.5 to 98.0 μ ; secondary sporangia with persistent spiny walls, many spored, mostly 35—42 μ diameter ; columellae of the large sporangia oval to pyriform, hyaline to faintly coloured, mostly 37.5—56 × 35—42 μ in diameter, columellae of the secondary sporangia globose to subsiventrally flattened 22.5—27.5 μ in diameter ; sporangiospores numerous, smooth to faintly roughened, thick walled, hyaline, globose, 6.25-7.5 μ in diameter, chlamydospores and zygospores not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The present species was isolated from the soil samples (pH 6.8—7.5 ; moisture content-13—15.5 per cent) collected from Agricultural Farm of Allahabad University. Culture deposited in the culture collection of the Botany Department as Mx—14.

2. *Absidia orchidis* (Vuill.) Hagem, Fig. 1—E—K.

Colonies on PDA and SMA at first white, later turning black on maturity ; mycelium more or less stoloniferous, at times curved towards the substratum to which their ends were attached by a tuft of rhizoids ; stolons branched sympodially and bearing sterile or fertile branches, the latter occuring singly or in groups of two or three. Sporangiophores simple or branched once only a short distance below the tip, the branch also ending in a similar sporangium ; a septum always present at a short distance below the sporangium. Sporangia ovate with an apophysis usually 42 × 28 μ, wall incrusted with fine granules, diffluent, leaving a small rigid collarette. Columellae conic, rounded, longer than broad 12.5—17.57 × 10—15.0 μ, usually having a knob at the tip, slightly constricted at the base. Sporangiospores, spherical slightly brownish, varying from 2·5—4 μ. No zygospores seen.

The present species was isolated from soil (pH 7.2, moisture content 13.2 per cent) samples collected from Agricultural Farm of Allahabad University. Culture deposited in the culture collection of the Botany Department as Mx—11.

3. *Circinella mucoroides* Sait. Fig. 2. A—C.

Colonies on SMA pale olive gray, turning at maturity brown in shade ; reverse pale smoke-gray to marguerite-yellow ; odour yeast like Sporangiophores 18 μ in diameter, arising from the substrate, weak, hyaline to light brown beneath the sporangium, in loose open spirals terminating mostly into 1 or 2 sporangia, sympodially branched, produce either sterile spines or circinate sporangia bearing stalks ; sporangia globose to slightly flattened, usually 50—80 μ but upto 85 μ in diameter, white then dark gray, sporangial wall smooth to slightly roughened, transparent ; columellae cylindrical, oval or nearly pyriform, 20—30 × 30—40 μ some upto 40 × 65 μ, collar always present, often small spine like, projections above, gray to light brown, sporangiospores globose to short oval, smooth, 3.5—6.0 μ in diameter, oval ones 5.25 × 7.5 μ. Zygospores and chlamydospores not known.

This species was isolated from saline soils of Lucknow District. Culture deposited in the culture collection of Botany Department as Mx—25.

4. *Circinella rigida* Smith. Fig. 2. A—D.

Colonies on SMA rapidly growing, pale neutral gray to light neutral gray, reverse olive-gray, odour none ; sporangiophores 5—10 μ in diameter, smooth, hyaline, with a septum beneath the sporangium, twisted, soon collapsing, bearing single sporangium on a branch never forming an umbel ; sporangia at first white turning at maturity, borne circinately, pyriform in shape, size 18.8 μ—70

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

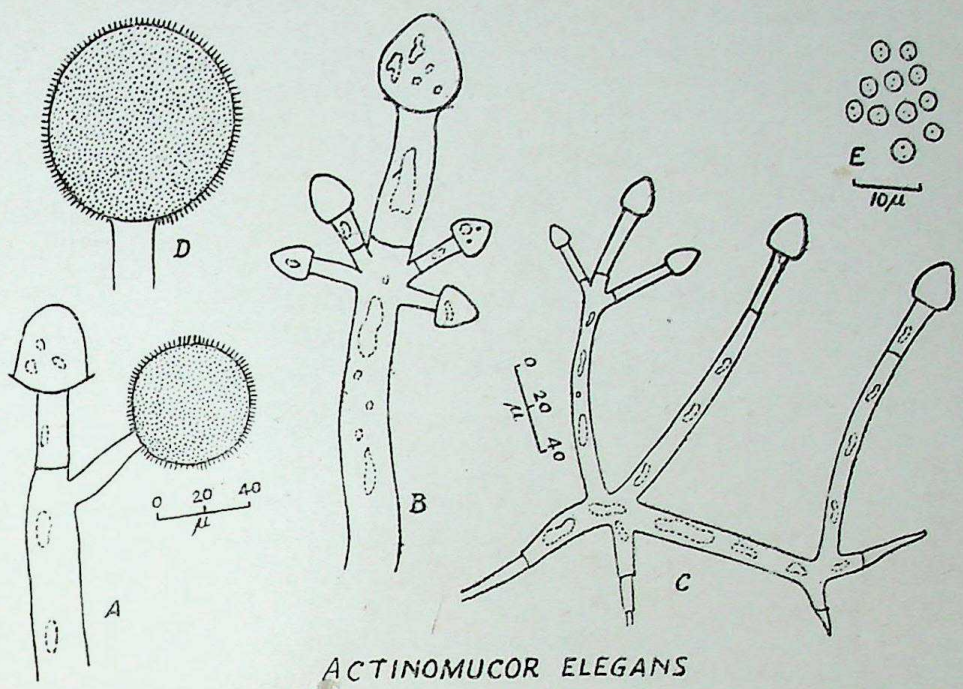
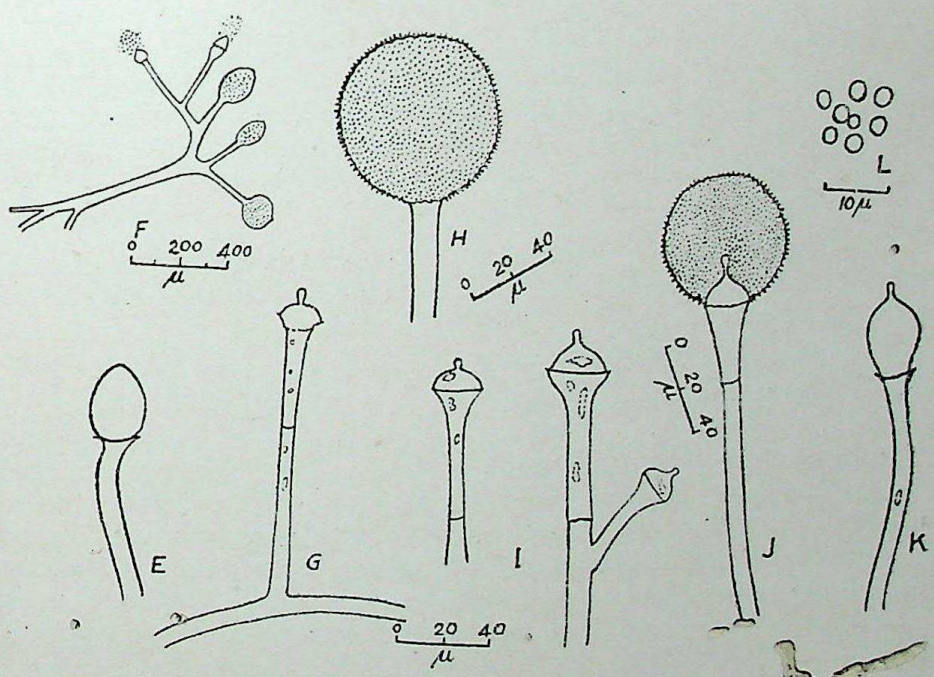


Fig. 1. A. Sporangiophore ; B. Branched Sporangiophore ; C. Stolon ; D. Sporangium ; E. Sporangiospores.



F. Branched Sporangiophore ; H. Sporangium ; E—K. Columella ; L. Sporangiospores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

diameter ; sporangial wall incrusted with granules, resistant ; columellae 20—36 μ in diameter, with an apophysis, dark gray with most of the sporangial wall attached, sporangiospores 3—6 μ, globose, smooth, light bluish black, chlamydospores present ; zygospores not seen.

This species was isolated from Dark gray soils of Jhansi, Bundelkhund. Culture deposited in the culture collection of the Botany Department as Mx—26.

5. *Mucor globosus* Fischer Fig. 3, A—D.

Turf white on PDA fast growing later turning yellowish brown with age, upto 20 mm. high, sporangiophores, erect, richly branched upto 20—22 μ in diameter, with oil drops in the mycelium, sporangia globose, yellowish brown, 70—120 μ (mostly 98 μ), wall diffluent, columellae free, brownish, oval to oblong or pyriform, 50—60 × 49—52 μ ; sporangiospores, globose to spherical, 4.0—7.5 μ ; chlamydospores present, mostly intercalary. Zygospores not seen.

This species was isolated from Chaubattia orchards, Ranikhet, Almora, from the maize field soil. Culture deposited in the culture collection of the Botany Department as Mx-27.

6. *Mucor rouxianus* (Calmette) Wehmer. Fig. 3,

Colony at first white about 4 m.m. in height, at maturity gray ; sporangiophores, branched sympodially ; sporangia, globose, 20—100μ (mostly 50 μ in diameter) ; columellae about 35-40 μ high, wall diffluent ; sporangiosphores 4.2-5.6 μ long sometimes upto 7 μ thick ; Gemmae present ; zygospores unknown ; chlamydospores not seen.

This species was isolated from Chaubattia orchards, Ranikhet, Almora, from brown forest soil. Culture deposited in the culture collection of the Botany Department as Mx-28.

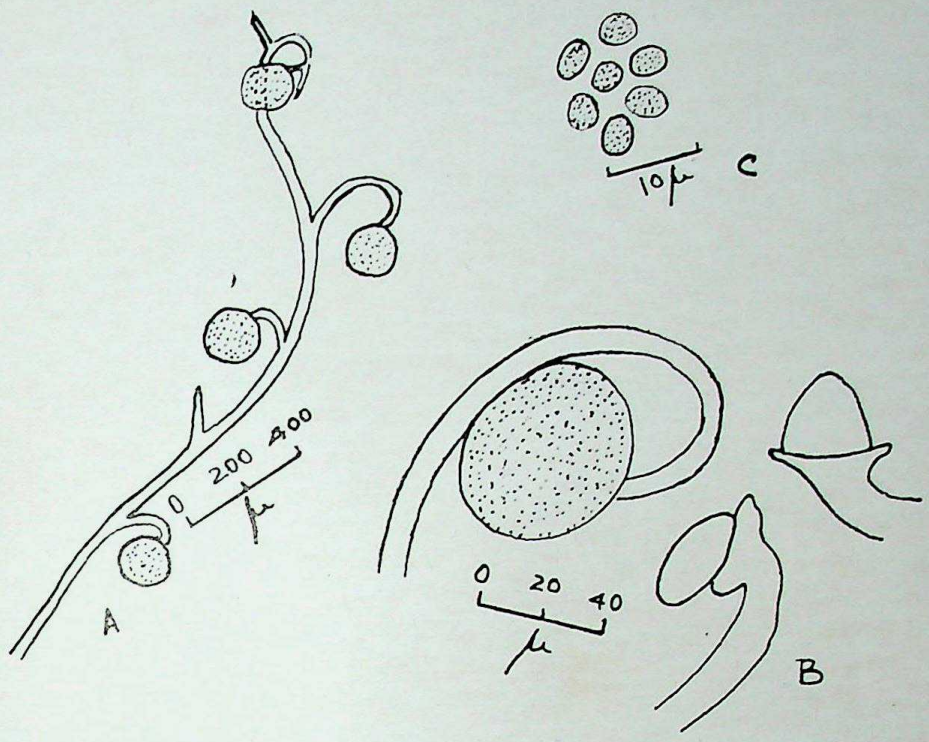
7. *Mucor lausannensis* Lend. Fig. 4 A to D.

Colonies on SMA and PDA at first yellow, later gray in colour, with sweetish smell. Sporangiosphores erect, 7-10 μ in diameter, simple or branched. Sporangia, spherical, 40-54 μ in diameter ; wall smooth, fragile, leaving a small irregular collarette ; columellae, spherical to oblong. Sporangiospores variable, oval to elliptical, 4-10 × 2-6 μ, average 8 × 6 μ, hyaline but yellowish in mass. Chlamydospores and zygospores not seen.

This species was isoloted from dog excreta in Allahabad. It being described fully for the first time from India by the author. Ajrekar and Ramaraju (1931) kept it under doubtful species.

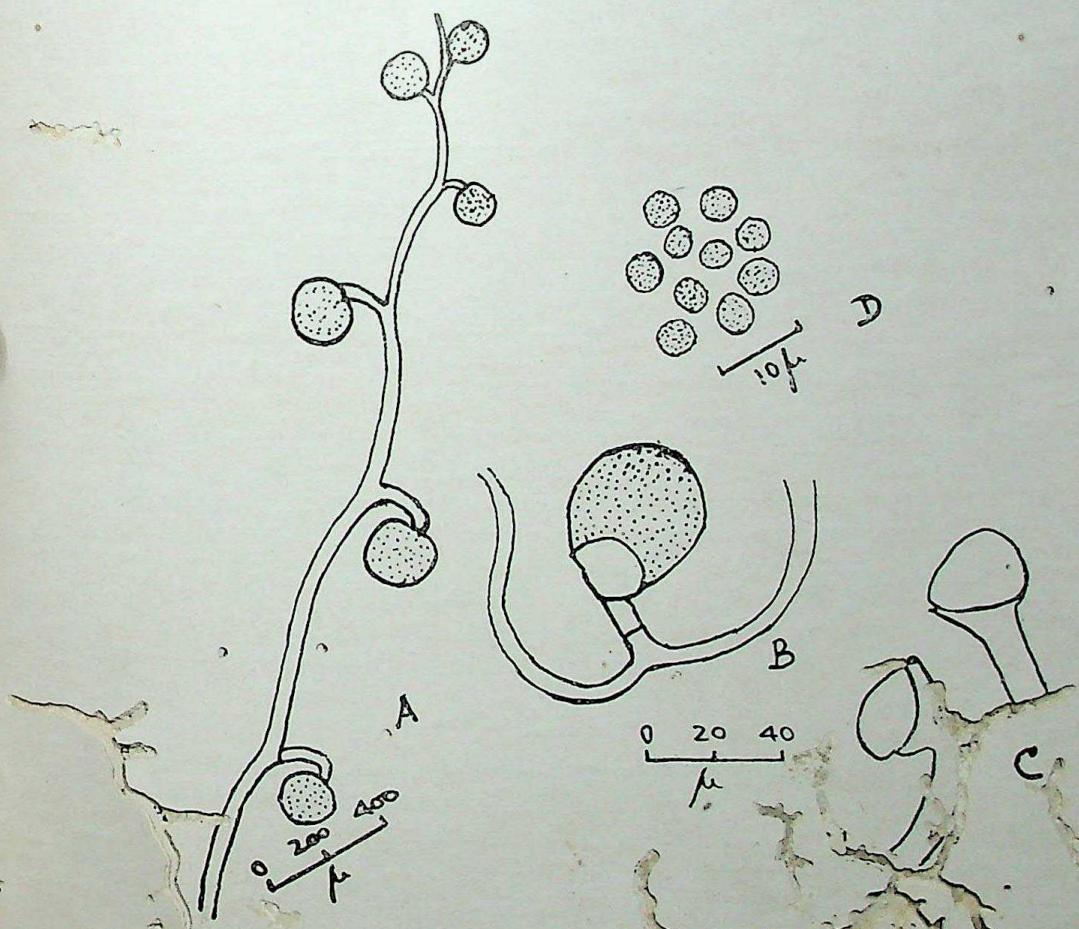
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



CIRCINELLA MUCOROIDES

Fig. 2. A. Sporangiophore ; B. Sporangium and Columellae ; C. Sporangiospores

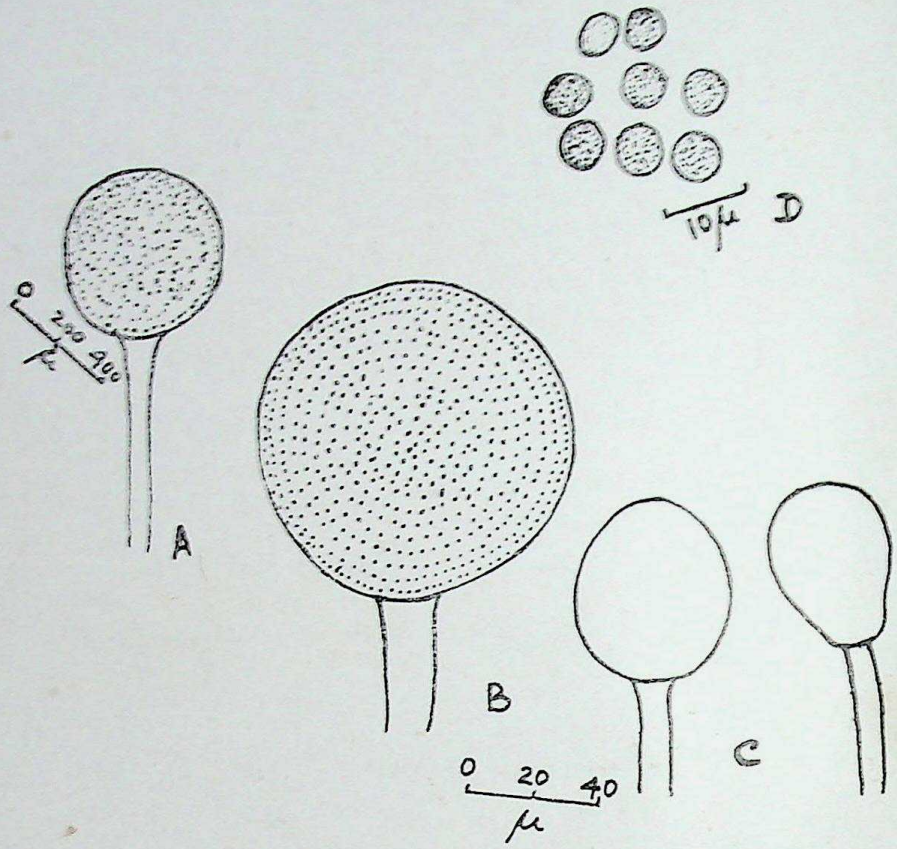


CIRCINELLA RIGIDA

A. Sporangiophores ; B. Sporangium ; C. Columellae ; D. Sporangiospores

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



MUCOR GLOBUSUS

Fig. 3. A. and B ; Sporangiophore and Sporangium ; C. Columella ; D. Sporangiosphore.

This species was isolated from garden soils of Allahabad Botany Department and also from the dung. Culture deposited in the culture collection of the Botany Department as Mx-29.

8. *Mucor abundans* Povah. Fig. 4 A to F.

Colonies on SMA and PDA at first yellow, later turning smoky-gray. Sporangiophores erect, 6-22 μ in diameter, at first simple, later branched, with a septum above point of insertion of each branch. Sporangium, globose or subglobose, at first yellowish, later brown to black, 56-80 μ in diameter ; wall diffluent, smooth, leaving a small collarette, columellae subglobose to pyriform, 30-40×25-36 μ, hyaline or grayish. Sporangiospores variable, oblong to ellipsoidal, 4.2-5.0×2.8-4.2 μ. Chlamydospores abundant, both intercalary and terminal with yellowish contents. Zygospores not seen.

The present species was isolated from Garden Soil of Allahabad University, Botany Department, and deposited in the culture collection of the Department as Mx-10.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

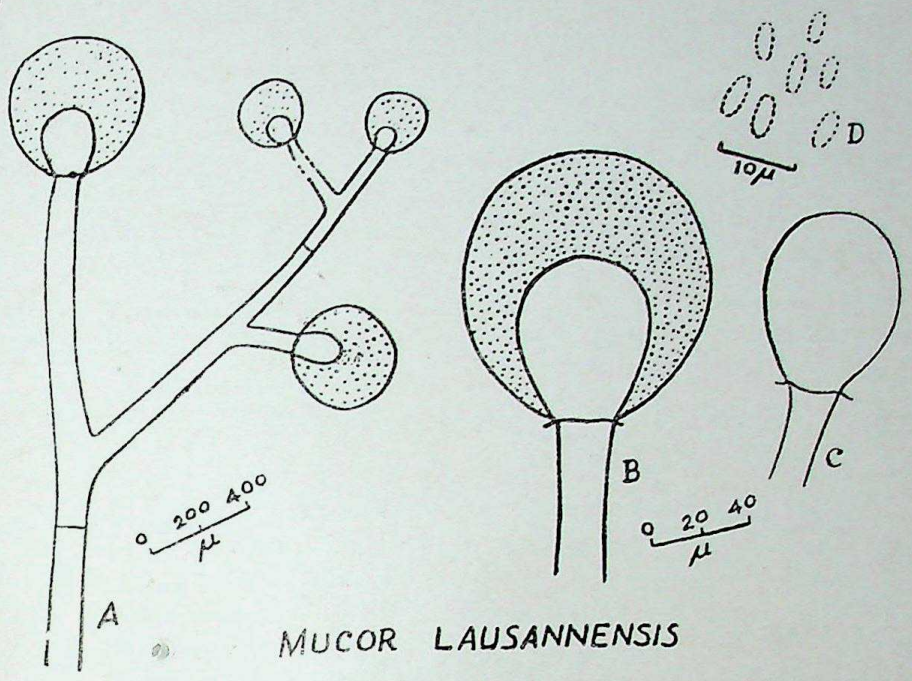
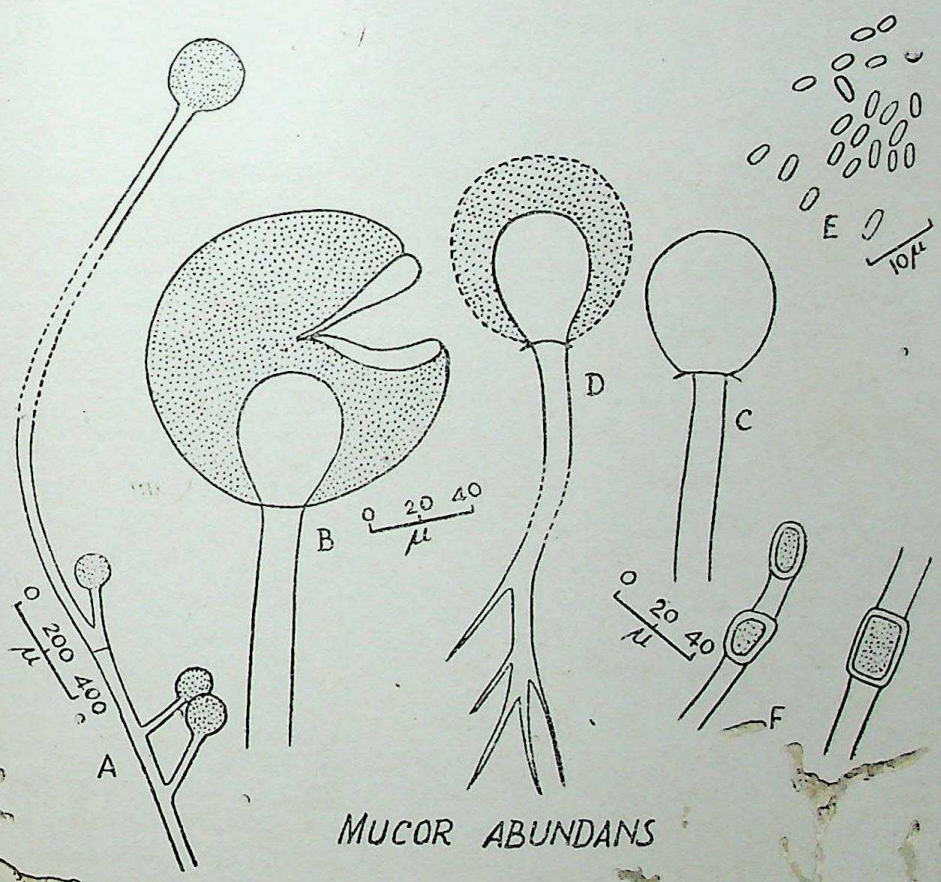


Fig. 4. A. Branched Sporangiophore; B. Sporagium; C. Columella; D. Sporangiospores.



A. Branched Spongiophore; B. Sporangium and rhizoids; D. Columellae; E. Chlamydospores (intercalary and terminal).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

9. *Mucor ramossissimus*. Hesseltine. Fig. 5, A—C.

Colony white on PDA, turning at maturity gray upto 20 m.m. in height, sporangiophores, erect, branched, branching just below the septa; sporangia, globular, persistant, yellowish brown, 60-80 μ in diameter (av. 63.5-76.2 μ); columellae, globose, 30-38 μ in diameter, without collarette, sporangiospores, elliptical to oval, 5-5.6 $\times$ 7-9 μ in size (av. 5.6 $\times$ 8.4 μ); Chlamydospores and zygospores not seen.

This species isolated from the soil near Kulbhaskar Ashram Agriculture Degree College, Allahabad, in the month of October. (C. C. Mx-31).

10. *Gongronella butleri* (Lend.) Pey. and D. Ves. Syn. *Mucor vesiculosus* Smith. Fig. 5 A-C

Colony white floccose on PDA, persistently white even at maturity reverse uncoloured; vegetative aerial hyphae long and stout 6-8 μ in diameter, sporangiosphores arising mostly as short branches usually with a septum below the sporangium; mostly unbranched but occasionally forming sympodial cymes; sporangia, globose with a subsporangial vesicle, 20-45 μ in diameter vesicle 7-15 μ in diameter; columellae hemispherical, 5.6-10 $\mu \times$ 7.8-15.6 μ, colourless; sporangiospores, oval, irregularly, 2-2.8 $\times$ 1.5-2.0 μ.

The present species abundantly found in Himalayan soils of Chaubattia orchards, Ranikhet, District Almora. Culture deposited in the culture collection of the Botany Department as Mx-32.

In addition to the species described above many other Mucorales have been isolated from these soils. These, although have been reported earlier, are of rare occurrence and include, *Absidia spinosa* Lend., *A. coerulea* Bain., *Circinella muscae* (Sorok.) Berl. and de Toni, *C. simplex* Van. Tiegh., *Rhizopus stolonifer* (Fr.) Lind, *R. arrhizus* Fisch., *R. homothallicus* Benj. (R. K.), *Mucor racemosus* Fres., *M. fragilis* Bain., *M. luteus* Linne., *M. mucedo* (Linne.) Bref., *M. genevensis* Lend., *M. janssenii* Lend., *M. spinescens* Lend., *M. hiemalis* Wehm., *Mortierella ambigua* Meh., *M. pussillus* Lindl. *Helicostylum piriforme* Bain., *Cunninghamella verticillata* Pain., *C. bertholletiae* Stad., *C. elegans* Lend., *Zygorhynchus heterogamus* Vuill., *Z. moelleri* Vuill., *Syncephalastrum racemosum* (Cohn.) Schrot. and *Phycomyces nitens* Agard.

SUMMARY

About fifty different species of the order Mucorales were isolated from various soils. Out of these nine were reported for the first time from Indian soils, *viz*, *Actinomucor elegans* (Eidam) Benj. and Hess., *Absidia orchidis* (Vuill.) Hagem., *Circinella mucoroides* Sait., *Circinella regida* Sitto., *Mucor globosus* Fischer, *Mucor rouxianus* (Calmette) Wehmer, *Mucor lausannensis* Lend., *Mucor abundans* Povah, *Mucor ramossissimus* Hesseltine, and *Gongronella butleri* (Lend.) Pey. and D. Ves., and the rest are either of common or of rare occurrence during various seasons.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

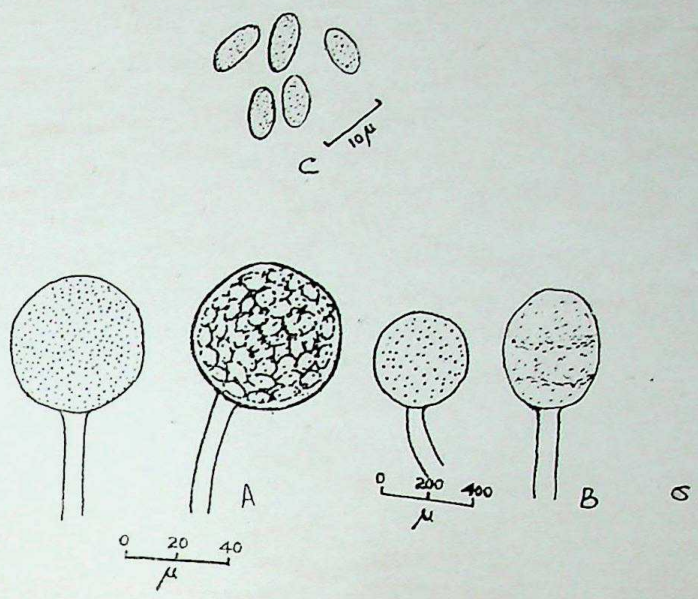
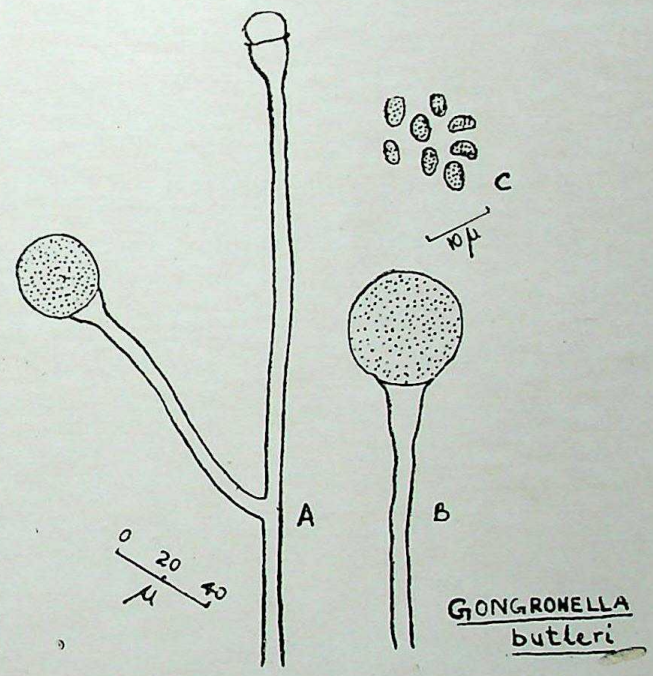


Fig. 5. MUCOR RAMOSSISSIMUS—A. Sporangia, B. Columella, C Sporangiospores.



GONGRONELLA BUTLERI—A. Branched Sporangiosp[illegible] with sporangia, B. Sporangium with subsporangial vesicle. C. Sporangiophore.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The author is grateful to Prof. R. K. Saksena, formerly Professor and Head of the Botany Department, University of Allahabad for his guidance and kind help during the course of present study and to Dr. B. S. Mehrotra, Assistant Professor, Botany Department, University of Allahabad for his help in identification of some of the isolates. Thanks are also due to Prof. R. N. Tandon, providing the Laboratory facilities.

Botany Department,

University of Allahabad,

Allahabad.

REFERENCES

AJRERKAR, S. L. AND K. DHARMARAJULU (1931) A study of the Mucorinae of the city of Bombay. *J. Indian bot. Soc.* **10** : 29-34.

GILMAN, J. C. (1957) A Manual of Soil Fungi. Iowa State College Press Journal.

NAUMOV, N. A. (1939) Cles des Mucorinees. Paul Lechevalier, Paris, 137 pp.

SAKSENA, R. K. AND B. S. MEHROTRA (1952) Fungus flora of an Allahabad Soil. *Proc. Nat. Acad. Sci.*, **22** : 22—43.

WAKSMAN, S. A. (1916) Do Fungi actually live in soil and produce mycelium. *Science*, **44** : 320—322.

WARCUP, J. H. (1950) The soil plate method for isolation of fungi from Soil. *Nature*, Lond., **166** :117.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

Inhibition of sporulation by light in Alternaria triticina Prasada and Prabhu—A. S. PRABHU AND R. PRASADA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—*Alternaria triticina* the causal agent of leaf blight of wheat[1] sporulated sparingly in artificial culture when kept in ordinary day light in the room ($23 \pm 2°$ C). However, it was observed that cultures which were kept in the incubator at 25° C sporulated heavily. Investigations were, therefore, undertaken to find out whether heavy sporulation in the incubator was in any way connected with the absence of light.

The single spore isolate of the fungus was grown on standard nutrient agar (K_2HPO_4 1.36 g ; Na_2CO_3 1.06 g ; $Mg\ So_4\ 7H_2O$ 5.0 g ; dextrose 5.0 g , asparagin 1.0 g ; agar 20.0 g ; to make one liter with distilled water) as it was found to be the best medium for sporulation. Also, the formation of aerial mycelium was much less in this medium and the spores could easily be separated from the surface. The source of light used was three daylight fluorescent tubes of 80 watt each arranged in a chamber in which the cultures were kept. The cultures were grown in petridishes containing 20 ml. of medium. After inoculation with the sterile fungus mycelium the petridishes were exposed to different intensities of light (50, 100, 150 ft. candles) obtained by keeping them at varying distance from the light source. Three plates were used in each treatment and the equal number of plates wrapped in black paper were kept side by side which served as control. Another set of plates alongwith the controls were placed in the temperature controlled room ($23 \pm 2°$ C) near the window where they were exposed to diurnal light and darkness. After incubation for 10 days radial growth measurements were taken along two diameters at right angles to each other in each petridish. The spores were removed by flooding the mycelium with water and the concentration of the spores was determined by using Haemocytometer slide with Neubauer ruling. A mean of 10 counts was taken and the results are presented in the table below.

TABLE : Effect of light on growth and sporulation of *Alternaria triticina* after 10 days.

Treatment	Average radial growth (mm.)	Sporulation (per ml.)
Continuous light of 50 ft. candles	55.0	Nil
Continuous light of 100 ft. candles	51.2	Nil
Continuous light of 150 ft. candles	52.9	Nil
Continuous darkness	55.7	80,000
Alternate light and darkness	53.5	36,000

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It can be seen from the table that there is no change in the rate of growth of fungus in any of the treatments. But sporulation was found to be abundant in petriplates wrapped in black paper, whereas in the plates exposed to different intensities of light there was no sporulation at all. In the case of petriplates exposed to diurnal light and darkness there was partial sporulation as shown by the fact that 36,000 spores per ml. were obtained as compared to 80,000 spores in petriplates exposed to total darkness.

Petriplates exposed to continuous light did not sporulate even after 20 days. After exposure to continuous light for 10 days the darkness of 48 hours was sufficient to induce sporulation all over the surface of the colony. On the contrary, when the colonies were exposed to continuous light after 10 days incubation in complete darkness the mycelium which was produced afterwards in light remained vegetative, suggesting the inhibitory effect of light on sporulation. Similar results were obtained when the experiment was repeated by using malt extract agar and potato dextrose agar showing thereby that there is no effect of the medium on sporulation.

Light is reported to be absolutely necessary for sporulation in many fungi. The sporulation of *Alternaria solani* is stimulated by sunlight[2]. On the other hand *Cephalothecium roseum* and *Sclerotinia fructicola* sporulate well in darkness, much less and not at all in light [3, 4]. Conidial production of *Alternaria brassicae var. dauci* isolated from wilted carrot leaves fall into two phases, sterigmata being formed only under the influence of fluorescent light, whereas an intervening period of darkness was requisite for the production of conidia[5]. *Alternaria triticina* is an unique example of a fungus which sporulates only in darkness and not at all in light. The fact that sporulation was inhibited in that part of the mycelium which was produced in light and the transfer of the culture to darkness later induced sporulation all over the colony suggests that some factor responsible for sporulation is either inhibited or not synthesized in the presence of light.

ACKNOWLEDGEMENT : Sincere thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for his keen interest in the progress of the work.

[1]Prasad, R. and A. S. Prabhu, *Indian Phytopath.*, **15** : 292-293 (1962).

[2]Rands, R. D. *Phytopathology*, **7** : 316-317 (1917).

[3]Hedgecock, G. C. *Ann. Rept.* Missouri. Botan. Garden. **17** : 115-117 (1906).

[4]Hall, M. P. *Ann. Bot.*, **47** : 543-578 (1933).

[5]Witsch. H. Von and F. Wagner., *Arch. Mikrobiol.*, **22** : 307-312 (1955).

To evaluate methods of applying 1, 2-di-bromo-3 Chloropropane for the control of nematode root-galls—R. B. S. BIRAT, Plant Protection Section, Department of Agriculture, Bihar, Patna. In a screening test with several nematicides, 1, 2 dibromo-3-Chloropropane (Nemagon) provided an excellent control of the root-knot nematodes, *Meloidogyne* spp. at the dosage 2 gallons per acre (Birat[1]). Since the conventional method of injecting the soil fumigant is time consuming on field scale, it

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

was considered expedient to evolve some convenient method of applying this nematicide. A micro-field trial was conducted during the year 1960 in the Entomological field Liboratory under the premises of the Bihar Agricultural College, Sabour. A nematode infested field (24′×20′) was divided into four equal blocks and each further subdivided into four identical plots of 5′×4′ dimension to accommodate four randomized treatments, replicated four times. Soil drenching, furrowing and injection were compared with non-fumigated control. In the injection method, the fumigant was injected 8″ deep 2″ apart in rows by the help of soil injector. The hole openings were closed with semi-dried soil immediately after the injection. The furrow method consisted of six to eight inches deep furrows at a distance of two feet apart. Requisite quantity of the fumigant was sprinkled in the furrows by the help of watering can after diluting it in tap water. The furrows were immediately covered with semidried field soil. In the soil drenching, the soil was stirred to a depth of nine inches by means of a spade and the fumigant was applied as surface drench mixed in water sufficient to wet the soil. Finally light irrigation in all the treated plots including the control was given in order to provide uniform seal over the entire experimental area against surface evaporation of the fumigant.

Fifteen days after the treatment, ten days old healthy tomato seedlings were transplanted in the plots at a distance of two feet in rows one and half feet apart. Fortyeight days after transplanting, three plants per plot per replication were uprooted randomly and examined for the presence of root-galls.

TABLE 1 : Effect of application methods on the formation of root-galls.

Application methods	Total no. of plants observed	Average no. of root-galls per plant
1. Soil drenching	12	17.2
2. Furrowing	12	5.4
3. Soil injection	12	0.4
4. Control	12	47.9

It is evident from the data presented above that all the treatments reduced root-knot incidence. The soil injection proved best of all followed by furrow treatment. Soil drenching seemed to be less effective possibly due to wide surface exposure, shallow penetration of the fumigant in the soil and consequent escape of the chemical during the process. The result is in conformity with the findings of Good and Steale[2].

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENT : Sincere thanks are due to Professor Dr. B. S. Lal, Head of the Division of Entomology, B. A. College, Sabour for his guidance and also to M/s Burmah Shell, Calcutta for providing 'Nemagon' for conducting the trial.

---

[1]Birat R. B. S. *Res. Found. Bull., B. A. College*, **4** : 45-46 (1961).

[2]Good, J. M. and A. E. Steale. *Plant. Dis. Reptr.*, **43** : 1099-1102 (1959).

A virulent Biotype of physiologic race 107 of Brown Rust of wheat: Puccinia recondita Rob: ex. Desm--R. PRASADA, S. K. SHARMA AND MADAN MOHAN, Division of Mycolgy and Plant Pathology, Indian Agricultural Research Insititute, New Delhi. It is obvious that physiologic races as icdentified with the help of standard differential hosts, can be further subdivided into biotypes with the help of additional varieties, and these are the actual units of such identification. Studies of the physiologic races and their biotypes of rusts are an important consideration for breeding rust resistant varieties. Generally, biotypes are more virulent than the races; many varieties found to be susceptible to the biotypes are resistant to their type races. Due to these facts, the identification and studies of biotypes needs more attention. To identify the physiologic races of brown rust of wheat, a set of 8 differential wheat varieties originally selected by Johnston and Mains[1] has been used here as in almost every other country. About the identification of biotypes of brown rust of wheat, not much work has been done in India. Prasada and Sreekantiah[3] in the case of stem rust of wheat, emphasised the need for the adoption of supplimentary differential hosts for identifying the biotypes within the races.

In the present studies, the rust collecitons of race 107, identified in different collections from widely separated localities, were studied on a large number of wheat varieties. For comparison, the type culture of race 107, which was originally picked in 1935 (Mehta[2]) from a rust collection received from Khanewal (Punjab) on wheat variety Pb. 8A and maintaind in the type culture collection, was used in these pathological tests.

Two rust collections of race 107, one collected from Vijapur (Gujrat) on wheat variety E. 875 and other from Meerut (U.P.) on local wheat, were taken for determination of biotypes. The types of infection produced by these collections on standard differential hosts were similar to those produced by the type culture of the race, but show differences in pathogenicity on 7 other wheat varieties.

The infection types of rust collections and type race in these 7 wheat varieties are given in table 1 for comparison.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : The types of infection produced by race 107, and its biotype on the 7 wheat varieties.

Sl. No.	Varieties	Type race 107	Vijapur E 875*	Meerut Local wheat
1.	Charter (E557)	0 ;	4	4
2.	Supremo (1847)	0 ;	3-4	3-4
3.	E 4874	0 ;-1	4	4
4.	E 5059	0 ;-2	4	4
5.	E 6013	0 ;-1	3-4	X
6.	E 6016	0 ;-2	4	4
7.	E 6034	0 ;	X	3-4

*S. S Culture from this isolate has been established to represent race 107 biotype A.

As the above table indicates, both the rust collections of race 107 from Vijapur and Meerut are very much similar to each other, but differ from the type culture of race 107. All the seven varieties were found to be susceptible to these collections but resistant to the type race. Thus this biotype is more virulent in its pathogenicity than the original race.

The biotype isolated here of race 107 is designated as 107A, and wheat variety Charter (E557) has been selected as supplementary differential host to identify this biotype.

ACKNOWLEDGEMENT : Grateful thanks are due to Dr. B. L. Chona, Head of the Division, for his keen interest and providing necessary facilities.

[1]Johnston, C. O. and E. S. Mains. U. S. Dept. of Agric. *Tech. Bull.* 313 (1932)

[2]Mehta, K. C. Further studies on cereal rusts in India. Sci. Monogr. 14. Indian Counc. Agric. Res. (1940)

[3]Prasada, R. and K. R. Sreekantiah. *Indian Phytoapath.* 9 : 209-211 (1956).

A new leaf-spot disease of Singhara (Trapa bispinosa) caused by Bipolaris tetramera —G. P. SINGH AND S. LAL.—Department of Plant Pathology, Government Agricultural College, Kanpur—A new leaf spot disease of singhara (*Trapa bispinosa* Roxb.) was first observed during the months of August and September, 1962 in most of the ponds near Kalyanpur, Kanpur. In subsequent years also, the crop was found to be seriously affected with this disease in some of the ponds. Repeated isolations from the diseased leaves

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

always yielded a culture of a *Helminthosporium* species. Since *Trapa bispinosa* is a popular and economically important rainy season pond crop in India and no species of *Helminthosoporium* has ever been recorded on this crop, it was considered desirable to study the above noted disease. The present paper describes briefly the symptoms and the causal agent of the disease.

The disease manifests itself as pin point sized mummy brown spots with a pale yellow margin which is clearly visible on placing the affected leaves in diffused sunlight. The spots are more distinct on the upper surface of the leaves. These spots gradually enlarge in size and become circular to oval or irregular in shape (Fig. 1). The mature spots usually measure from 2–7 mm. in diameter. As the disease advances, some of the spots coalesce to form larger patches of dead areas on the affected leaves. The fructifications of the pathogen develop conspicuously over the larger and older spots which often become thinner and lighter in the centre.

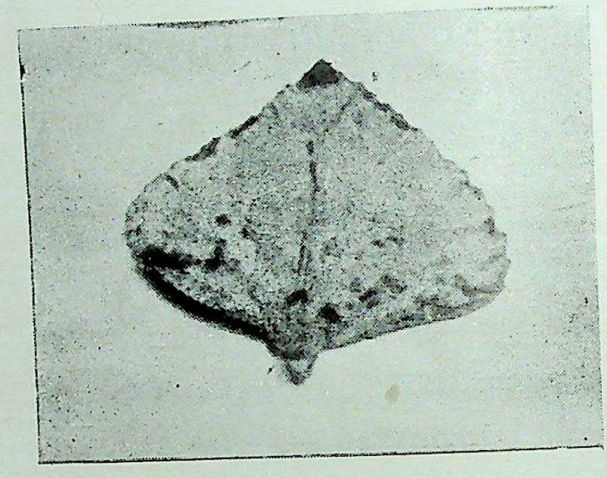


Fig. 1. Spots on a leaf of 'Singhara' inoculated with *Bipolaris tetramera*.

The colour of the fungus colony on potato dextrose agar (PDA) was light brown during the early stages of growth, but became dark brown to black in colour with age. The mycelium consists of septate, olivaceous and profusely branched hyphae which measure 3 to 8μ in diameter. The conidiophores are simple or compound, dark olivaceous and septate, the septa being 10 to 42μ apart. They emerge either through the stomata or through the epidermis of the upper leaf surface and bear conidia in clusters at irregular intervals. The conidia are usually 4-celled, dark olivaceous to brown, straight, elongated and elliptical tapering towards the rounded ends, (Fig. 2). They measure 20–28 × 10–14μ on host and 20–38 × 10–20μ in culture. They germinate by a germtube from each polar cell.

The fungus produced a number of sclerotia in old cultures. The sclerotia are simple or branched, and elongated, and measure 2–6 mm. in length and 100–650μ in width. The sclerotia are composed of hard

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

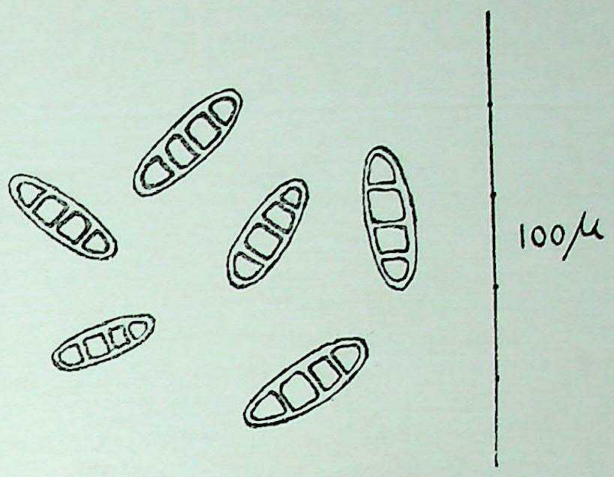


Fig. 2. Conidia.

hyaline pseudoparenchymatous tissues surrounded by an outer black layer or rind. Many hyphal outgrowths arise from these rinds but they do not produce conidia.

The morphological characters of the present isolate appear to be similar to those of *Helminthosporium tetramera* Mckinney. However, in respect of septation, shape and attachment of conidia, the fungus greatly resembles *Brachycladium spiciferum* but clearly differs from it in respect of size of conidia and formation of sclerotia. The conidia of the present isolate are relatively larger in size and their measurements are in close agreement with those of *H. tetramera*. The present isolate also forms elongated simple or branched sclerotia which are never formed by *B. spiciferum*. Similar sclerotia have also been reported by Mckinney[1] in the cultures of *H. tetramera* and this character has been employed by Sprauge[3] to separate *H. tetramera* from *B. spiciferum*. In sclerotia formation as well as other morphological details the present isolate resembles *H. tetramera* which has been renamed recently as *Bipolaris tetramera* on the basis of germination of conidia by a germ tube from each polar cell. Similar mode of conidial germination from the polar cells has also been observed in the present isolate. The causal agent of the above noted leaf spot disease of singhara (*Trapa bispinosa*) is, therefore, identified as *Bipolaris tetramera* (Mckinney) Shoemaker.

ACKNOWLEDGEMENT : Thanks are due to Dr. H. K. Saksena, Professor of Plant Pathology, Government Agricultural College, Kanpur for useful suggestions and for going through the manuscript

---

[1]Mckinney, H. H. *U. S. Dept. Agr. Bull.* **1347** : (40) 1925.

[2]Shoemaker, R. A. *Canad. J. Bot.* **37** : 879-887 (1959).

[3]Sprauge, R. The Ronald. Press Company, New York (1950).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

"Purple leaf" disease of beet:—S. P. CAPOOR AND D. C. SHARMA*—Indian Agricultural Research Institute, Plant Virus Research Laboratory, Poona-5—During the year 1958, a previously unrecorded virus disease of beet, *Beta vulgaris* L., was collected from Balmukundnagar Farm in Poona, and briefly discussed in 1961 before the Indian Science Congress Association.[1] Studies of the symptomatology, host-range, and physical properties of the virus showed that it was related to TMV. The virus culture for these tests originated from infected beet plants collected from various fields in Poona and was maintained in beet.

The number of infected plants usually ranged from 10 to 25 per cent, but higher incidence of the disease was common in Balmukundnagar Farm and its neighbouring fields. Symptom expression in the field crop varied with age of plants. Severely infected plants were stunted and leaves had a tendency to stand erect and come closer, unlike the healthy plants, where the leaves are broad, long and profuse. Leaves of infected plants showed an unusual intense purple colour, while the young emerging leaves showed it prominently. Few leaves developed minute necrotic lesions all over the lamina. These lesions were distinct from the necrotic spots caused by *Cercospora* sp., which are comparatively larger and very prominent. The symptoms described above are most common during December-February at Poona. Subsequently, as the plants mature, there is a tendency towards recovery.

On experimentally inoculated beet plants the virus causes mild chlorotic spots, followed by the development of a clear purple ring within 7 to 10 days of inoculation. Some days later, the whole of the affected leaf gets puckered and stands erect. The entire leaf blade gets

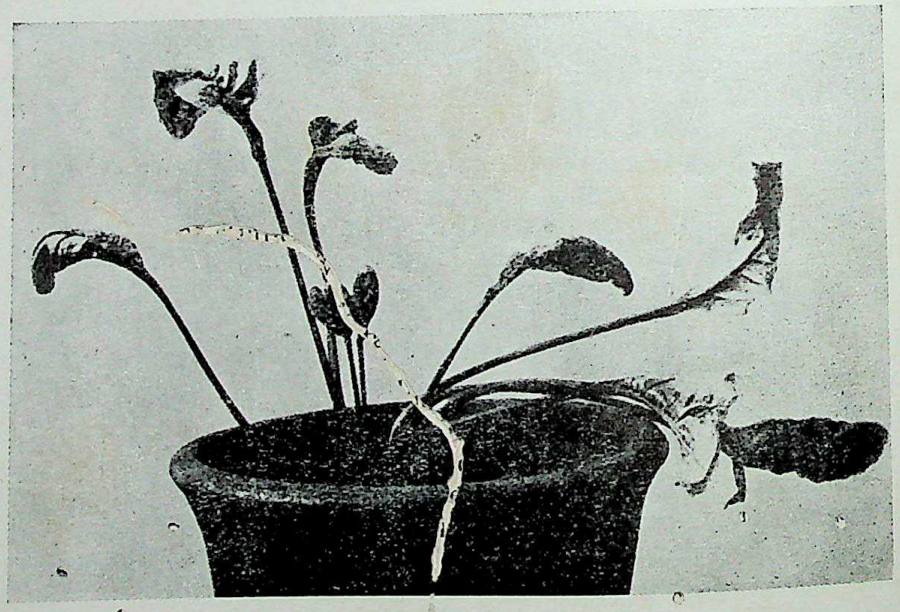


Fig. 1. "Purple leaf" affected beet plant showing leaf rolling and coloration of leaves.

*Present address: Resources and scientific Research Division, Planning Commission, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

rolled up and the young developing leaves become intense purple in colour and brittle in texture (Fig. 1). Young plants of beet are readily infected by the virus. Symptoms are masked during growth at higher temperature.

Several plant species were sap-inoculated using carborundum powder as abrasive. The virus produced local necrotic lesions on *Cassia tora* L.,[2] *Nicotiana glutinosa* L., similar to those produced by the type strain of tobacco mosaic virus and on *Chenopodium amaranticolor* L., *C. album* L. and *G. globosa* L. In *Nicotiana tabacum* L. var. White Burley, *N. sylvestris* Spegaz. and Comes, *Lycopersicon esculentum* Mill., and also in *G. globosa* mild mosaic symptoms appeared. *Spinacia oleracea* L. showed chlorotic rings on in oculated leaves followed by yellow vein-banding in leaves developing subsequently.

None of the following plant species tested were infected:

Datura inoxia Mill., *D. stramonium* L., *Crotalaria juncea* L., *Dolichos lablab* L., *Phaseolus vulgaris* L. var. Local, *P. vulgaris* var. Early Golden Cluster, *Pisum sativum* L., *Cyamopsis tetragonoloba* (L.) Taub., *Vigna sinensis* Savi, *Zinnia elegans* L., and *Triticum aestivum* L.

The physical properties of the virus were tested by the usual conventional methods on *N. glutinosa* as test plant and beet as source plant. The results showed that the virus withstood heating for 10 minutes at 90°C but not at 95°C, tolerated dilution upto 1 in 10^5 but not at 1 in 10^6 and stood ageing for over a month.

The above data show that the beet purple leaf virus (BPLV) causes characteristic symptoms in *Nicotiana tabacum*, *N. glutinosa*, and *Cassia tora* similar to those produced by the type strain of tobacco mosaic virus, withstands heating at high temperature and dilution as high as 1 in 10^5. These facts show that the disease in beet is caused by a strain of TMV which does not infect bean, but all the same systemically invades beet. The natural infection of beet by a strain of TMV and also its ability to infect it systemically has been shown for the first time. TMV is known to produce only local infection in beet as well as in sugar beet[3]. Other sap transmissible viruses that affect beet in nature or under experimental condition are (i) beet curly-top[1] which is sap-transmissible with difficulty, (ii) beet latent virus[5] which occasionally produces local lesions in beet leaves, (iii) beet mosaic[3], beet ring spot[6], and beet yellows[7], all of which produce only dark coloured necrotic lesions in beet. All these viruses are distinct from the "purple leaf" virus dealt with in this note in more than one respect. The strain identity of the purple leaf virus will depend on further detailed investigations.

---

[1]Capoor, S. P. and D. C. Sharma. *Proc. 48th Indian Sci. Cong.*, Part III: 500-501. (1961).

[2]Capoor, S. P. *Curr. Sci.* **30**: 353. (1961).

[3]Smith, K. M. *A Text-book of Plant Virus Diseases.* J. and A. Churchill Ltd. London. (1957).

[4]Bennett, C. W. *J. agric. Res.* **50** : 211-214. (1935).

[5]Smith, K. M. *Nature (Lond.)* **167** : 106[illegible]. (1951).

[6]Harrison, B. D. *Ann. appl. Biol.* **45** : 462-472. (1957).

[7]Bennett, C. W. *U.S.D.A. Tech. Bull. No.* 1218 : 1-63. (1960).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Two Powdery Mildews from Sikkim—J. N. KAPOOR, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12.—This note presents an account of 2 new species of *Erysiphe*. The specimens of these were collected during a mycological survey of Sikkim State during April 1962. *Erysiphe rhododendri* was collected from a *Rhododendron* forest, on way to Yumthang in northern Sikkim. This place is about 11,500ft. above the sea level and is snow-bound during the greater part of the year. The other species, i. e. *Erysiphe symploci*, was commonly found at altitudes ranging between 5000-7000ft. above the sea level, throughout the Sikkim State. The Type specimens of these species have been deposited in the *Herbarium Cryptogamae Indiae Orientalis*, New Delhi and are designated in the text by the H. C. I. O. numbers.

Erysiphe rhododendri sp. nov.

Hypophylla, efformans maculas alba vel sordide albas circularis, quae evadunt irregulares: mycelium persistans, textura implicata, crassum dense intertextum, efformans subiculum tenue cui perithecia insidunt dispersa, nigra circularia discoidea 100–120μ, cellulis distinctis polygonalibus; appendices sparsae et breves horizontaliter patentes intertextae mycelio simplices hyalinae ad 80μ longae; asci 8—9 in singulis ascocarpi, ovati, tum evadentes obovati cum sporae maturitatem attingunt, 60–64×26–30 μ; ascosporae 7–8 in singulis ascis, oblongo-ellipticae, unicellulares, hyalinae, contentis densis protoplasmicis, 20–24×14–16μ.

The mildew produces dirty-white circular patches, which become irregular as they enlarge. The mycelium is persistent, felty, thick and densely interwoven, superficial, forming a subicle in which perithecia are seated. Perithecia gregarious, black, circular, disc-like, 100—120μ in diameter; wall composed of distinct, polygonal cells. Appendages sparse, short and unbranched, 30–80μ in length, always spreading horizontally and are interwoven with mycelium; asci 8–9 in each ascocarp, ovate becoming obovate with short twisted stalk arising from the same base in fan like manner, double walled, 60–64×26—30μ in size; ascospores 7–8 in each ascus, oblong-elliptic. Single-celled hyaline, measuring 20–24× 14—16μ with dense protoplasmic contents (Fig. 1,2).

On living leaves of *Rhododendron* sp. (Ericaceae), on way to Yumthang (N. Sikkim) 19-4-1962. J. N. Kapoor, H.C.I.O. No. 27849.

No conidia could be observed in this specimen. As the mycelium is entirely superficial, it obviously belongs to *Erysiphe*. No *Erysiphe* species is recorded on any *Rhododendron* species from India. There is a solitary record of *Erysiphe polygoni* on *Rhododendron nudiflorum* and *R. occidentale* from U. S. A.[1] The Sikkim specimen is quite distinct from *E. polygoni* in having 7–8 ascospores in each ascus, whereas in *E. polygoni* there are only 3–6 ascospores. The Sikkim specimen is very characteristic in having thick, felty and densely interwoven mycelium, which forms a subicle. It is therefore, being proposed as a new species.

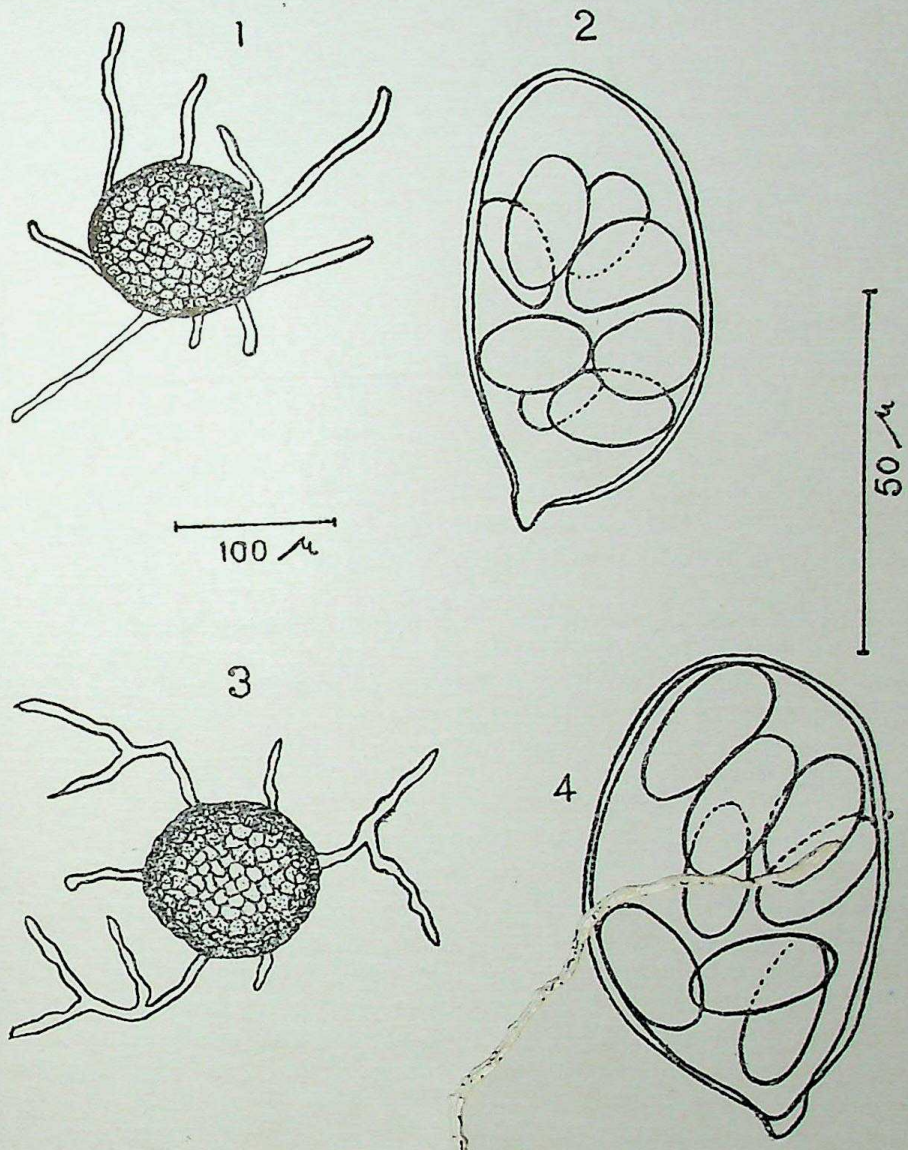
[1]Weiss, F., *Plant Dis. Reptr.*, Special Publication No. 1 part II: 317, (1950.)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Erysiphe symploci sp. nov.

Hypophylla, efformans maculas albas circulares vel irregulares, areis oppositis in pagina superiore chloroticis; mycelium evanescens; perithecia globosa dispersa fusce brunnea cellulis distinctis polygonalibus; appendices and 200μ longae, 4 latae, hyalinae, saepe furcatae, intertextae mycelio; asci bini vel terni (vulgo terni) in singulis ascocarpis, obovati vel subglobosi, 22–24×26–28μ; sporae 8, ovato-ellipticae, hyalinae, 22–24×4–6μ.



The fungus produces white powdery, circular to irregular patches on the under surface of leaf. The corresponding upper surface becomes chlorotic; mycelium thin, hyaline, superficial, evanascent; Cleistothecia globose, scattered, dark brown, cells of the wall distinct, polygonal; appendages very long, upto 150—200μ in length and 4μ in breadth, hyaline, often branched; asci 2–3 (mostly 3) subglobose to

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

obovate and measure 22–24×26–28μ; Ascospores 8, ovate-elliptic, 22–24×4–6μ, hyaline with dense protoplasmic contents (Fig. 3, 4).

One leaves of *Symplocos racemosa* Roxb. (Symplocaceae), Sribadam (W. Sikkim), 7-4-1962, J. N. Kappor, H.C.I.O. No. 27850.

This clearly belongs to *Erysiphe* as there is no internal mycelium. No conidia were present on the specimen. As no species of *Erysiphe* is recorded on this host genus or family, this is being proposed as a new species.

ACKNOWLEDGEMENT : Sincere thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, and Shri R. L. Munjal, Systematic Mycologist, for their keen interest and encouragement. I am also grateful to Rev. Father Dr. H. Santapau for rendering the latin diagnosis and to Sh. B. P. Agarwal for help in making the illustrations.

An undescribed Myxomycete from the coffee estates of Mysore.—V. AGNIHOTHRUDU, Rallis India Limited, Bangalore 1.—In the course of synecological investigations on the so-called 'New Malady' of coffee in mysore, the writer has been coming across several interesting micro- and macrofungi. The dense overhead jungle canopy of the coffee estates and the decomposing vegetable debris underneath the coffee bushes constitute a very ideal environment for the multiplication of several myxomycetes particularly during the wet weather.

In this brief paper, is presented a hitherto undescribed member of the genus *Licea sensu* Schrader *emend* Rostafinski, belonging to the order Liceales. This fungus was first recorded in the Ossoor Coffee Estate in the Hassan district of Mysore state. Later, a similar collection was made from Biccode Estate in the same district. The species is named *Licea mysorensis* sp. nov.

Licea mysorensis sp. nov. Agnihothrudu :

Plasmodium pallide vel sordide roseum, aqueum; sporangia dispersa vel gregaria, sessilia, globosa, nitenter cinnabarina cum juvenilia, evadentia alte alutaceo-brunnea vel fere nigra ad maturitatem, ad 3 mm. diam.; peridium coriaceum, leve, dehiscens per porum irregularem; sporae sordide albae in massa, pallide olivaceo-virides vel fere hyalinae sub luce transmissa, globosae, pariete sculpto verrucis obtusis, 5-7.5 (7) μ; pseudocapillitium raro adest.

Plasmodium pale pink, watery; sporangia scattered to gregarious, sessile, globose, bright cinnabar red when fresh, turning deep tannous brown to almost black with maturity, upto 3 mm. diam.; peridium leathery, smooth, dehiscing by an irregular pore; spores dirty white in mass, pale olivaceous green to almost hyaline in transmitted light, globose, the spore wall being sculptured with blunt warts, 5-7.5 (7) μ; pseudocapillitium rarely present.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

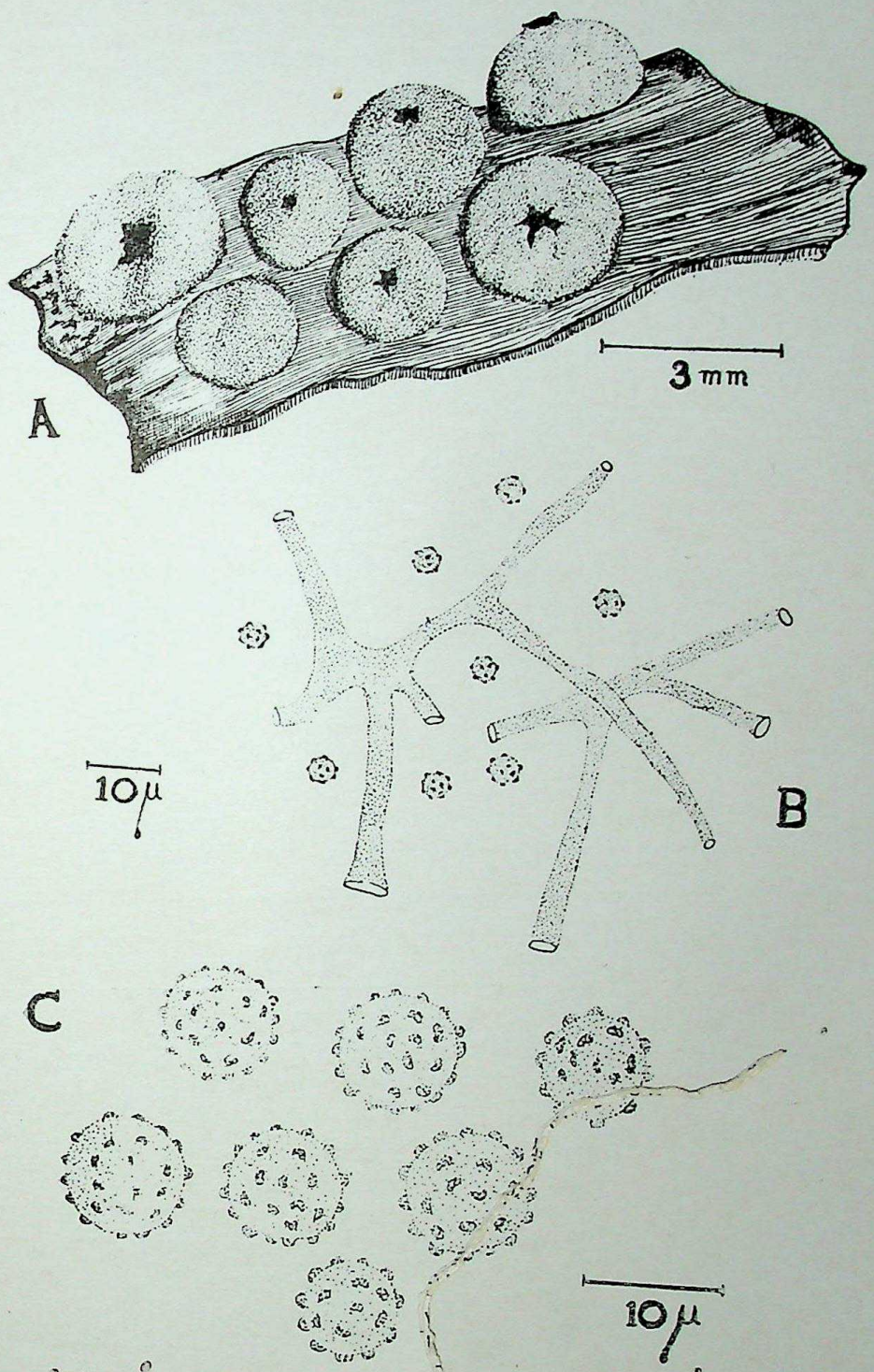


Fig. 1. *Licea mysorensis* Agnihothrudu from MYSP Herb. No. 750.

A. Sporangia on the bark of coffee showing the mode of dehiscence.

B. Pseudocapillitum and spores.

C. Spores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Type collected on the bark of coffee (*Coffea arabica* L.) from Ossoor Coffee Estate, Saklespur, Hassan district, deposited in the Mycological Herbarium of the State Agricultural College, Hebbal, Bangalore, under the MYSP Herbarium No. 750.

The closest ally to this species is *Licea castanea* G. Lister (Martin[1]), but the latter differs in having smaller sporangia, larger spores (8-10 μ) which are smoother. Besides, in *L. castanea* dehiscence is along definite lines to form plates or lobes.

ACKNOWLEDGEMENT : I am thankful to the Divisional Manager, Fertiliser and Pesticides Division, Rallis India Limited and the Special Coffee Research Association for their encouragement and facilities given and to Prof. Rev. Fr. H. Santapau, Director, Botanical Survey of India, for rendering the diagnosis into Latin.

[1]Martin, G. W. *North American Flora* Vol. 1 Part I. (1949)

Relative efficacy of some new fungicides for the control of hill bunt of Wheat :—J. S. GREWAL, P. C. JOSHI, K. D. PATHAK AND S. B. MATHUR (Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Fungicides are being used for the control of hill bunt of wheat (*Tellitia foetida* (Wallr.) Liro.) since long. This paper reports the results of experiments conducted with some new fungicides. Wheat seeds (variety N. P. 809) were artificially inoculated and treated with

TABLE 1 : Effect of seed-dressing fungicides on germination, disease incidence and yield of wheat.

Fungicides and Active Ingredient	Dosage in per cent	1962-63			1963-64		
		Percentage Germination	Disease incidence	Yield per acre (kg.)	Percentage Germination	Disease incidence	Yield per acre (kg.)
1	2	3	4	5	6	7	8
Agrosan GN (tolylmercury acetate)	0.20	39.3 (38.8)*	3.2 (9.9)*	471	35.4 (36.4)*	3.5 (10.3)*	381
Fusariol (ethylmercury cyanide)	0.20	41.5 (40.1)	1.0 (5.0)	495	40.1 (39.3)	1.4 (5.7)	340
Ceresan (Phenylmercury acetate)	0.20	42.5 (40.1)	Nil (0.6)	555	42.2 (40.5)	Nil (0.6)	344

Note : *The figures in brackets indicate percentages in transformed values.

Contd.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Contd.

1	2	3	4	5	6	7	8
Puraseed (n-phenyl mercuri-formamide, anilinocadmium lactate)	0.20	41.0 (39.8)	2.0 (5.1)	477	35.7 (36.6)	1.6 (6.2)	287
Quinolate-15 (Copper oxinate)	0.20	38.9 (38.6)	4.2 (11.1)	426	37.1 (37.5)	5.4 (13.4)	218
Copper Carbonate (copper carbonate)	0.30	42.6 (40.6)	1.0 (4.4)	469	40.8 (39.6)	1.9 (7.5)	318
Agrosan 5 W (organically combined mercury)	0.06	42.0 (40.4)	0.8 (3.9)	469	38.5 (38.4)	0.6 (3.4)	223
2 % Ceresan (ethylmercury chloride)	0.20	40.6 (39.6)	0.5 (2.7)	393	39.3 (38.8)	0.5 (3.2)	337
Granosan (ethyl-mercury chloride)	0.20	38.4 (38.3)	1.2 (5.6)	490	38.3 (38.2)	1.2 (6.2)	392
Leytosol B(phenyl-mercury urea)	0.06	40.1 (39.3)	2.2 (8.6)	489	39.4 (39.1)	4.4 (11.7)	301
Tritisan (penta-chloronitro-benzene)	0.20	44.4 (41.8)	0.1 (0.9)	483	40.2 (39.4)	0.4 (2.6)	501
Panogen [cyno (methyl mercuri) guanidine]	2 ml./kg.	41.0 (39.8)	Nil (0.6)	587	41.9 (40.4)	Nil (0.6)	591
N. I. Ceresan (ethyl-mercury phosphate)	0.06	41.0 (39.8)	2.2 (8.6)	483	41.6 (40.1)	3.3 (10.2)	499
Fernasan [bis (dimethylthio carbamoyl) disulphide]	0.30	41.7 (39.6)	2.7 (9.3)	481	38.9 (38.5)	2.0 (8.0)	442
Control (untreated)	...	40.8 (39.6)	45.0 (42.0)	195	37.2 (37.6)	63.0 (52.7)	163
Significance of "F" test	Not significant	Highly significant	Highly significant	Highly significant	Highly significant	Highly significant	Not significant
S. E. (d)	—	(2.55)	66.7	(6.76)	(1.95)		—
C. D. at 5 % level	—	(5.15)	134.8	(13.65)	(3.94)		—

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

fungicides as described by Grewal et al.[1] Experiments were conducted by randomized block design in 8′×5′ plots for a period of two years at Central Potato Research Institute, Simla with four replications for each treatment. The data regarding germination, incidence of disease and Yield were analysed statistically and results are summarized in Table 1.

It is evident from the data in Table 1 that none of the fungicides affected the germination adversely. Panogen and ceresan gave complete control of the disease with corresponding increase in yield. Statistically there was no significant difference in disease incidence recorded on Ceresan, Agrosan 5w, 2 % Ceresan, Tritisan and Panogen. The cost of seed treatment by panogen and Ceresan works out to be 40-50 *paise* per acre. It is, therefore, recommended that these two fungicides should be used for the control of hill bunt wheat.

---

[1]Grewal, J. S., Dharam Vir and P. C. Joshi. *Indian Pyhtopath.*, **15** : 287-289 (1962).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Deodar fruit extract—an inhibitor of chilli mosaic virus:—S. P. RAYCHAUDHURI AND K. C. CHADHA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—During the past few decades much work has been done on inactivation of plant viruses by plant extracts and attempts have been made to obtain plant virus inhibitors from higher plants. Allard[1,2] reported the inactivation of tobacco mosaic virus (TMV) by pokeweed and later several similar results have also been reported by various workers in respect of other viruses as well. Kassanis and Kleczkowski[3] identified the inhibitor from pokeweed as glycoprotein. Raychaudhuri[4] reported inhibition of a number of plant viruses by plant extracts.

Pine oil is known to be inhibitor for various species of fungi and has been used in fungicidal preparation. Wecratane[6] reported the inhibition of TMV infectivity by extracts of pine. The inactivation varied from 90-100 per cent as measured by local lesion assay method on half leaves of *Nicotiana glutinosa* L. Raychaudhuri and Chadha[5] reported that deodar fruit extract inhibited chilli mosaic virus (Ch MV).

For our experiments, deodar *Cedruds deora* (Roxb. Loud.) cones were collected from the Simla Hills in the month of May, 1964 and Ch MV was obtained from virus culture collection maintained at Indian Agricultural Research Institute and was multiplied on *Nicotiana tabacum* L. var. Harrison's special.

To obtain deodar fruit extract, 5 grams of material was crushed in pestle and mortar, adding 10 ml. of distilled water. Crude extract was strained through muslin cloth and centrifuged at 3,000 r.p.m. for 30 minutes in order to remove extraneous materials. To prepare the virus extract infected leaf material was crushed in pestle and mortar adding one ml. of distilled water per gram of leaf material and few drops of 0.5 per cent sodium sulphite. The extract was later strained through muslin cloth and finally centrifuged at 3,000 r.p.m. for 30 minutes.

In order to study the inhibition, one ml. of Ch MV preparation was mixed with three different quantities (0.5 ml., 1 ml., and 2 ml.) of deodar cone extract and was allowed to react for 15 and 30 minutes, before finally inoculating the local lesion host, *Chenopodium amaranticolar* L. by half-leaf technique. For each treatment, 20 half-leaves in five test plants were inoculated and the experiment was repeated three times.

In all the treatments, hundred per cent inhibition of the virus was observed. No lesion was developed on the inoculated half leaves, whereas on the untreated ones (control) 70-125 lesions appeared after

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

about a week. It is, therefore, obvious that deodar cone extract acts as an inhibitor of Ch MV

Thanks are due to Dr. B. L. Chona, former Head of the Division of Mycology and Plant Pathology, for his interest in this work.

---

[1]Allard, H. A. *U. S. D. A. Bull.* No. 40 (1914).

[2]Allard, H. A. *Phytopathology*, **8**: 51 (1918).

[3]Kassanis, B. and A. Kleczkowski. *J. Gen. Microbiol.*. **2**: 143 (1948).

[4]Raychaudhuri, S. P. *National Inst. Sci. India. Bull.*, **24**: 143 (1962).

[5]Raychaudhuri, S. P. and K. C. Chadha. *Ind. Sci. Cong.* Part III 574 (1965).

*[6]Wecratane, V. *Dis. Abstr.*, **22**: 1373 (1961).

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Reviews

Symptoms of Virus Diseases in Plants by L. Bos, 132 pp, published by Centre of Agricultural Publications and Documentation Wageningen, Netherlands. Second impression. Price Dfl 11. 50/23S/3.50, 1964

In this little book the author chiefly aims from the biologist's point of view to classify the histo-morphological changes brought about owing to viral infection. These days the attention of most workers is centred around on the structural biochemistry of the viruses and biochemistry of the infection process rather than on their pathological effect. In this changing trend of modern outlook the author attempts to emphasize the symptoms of virus diseases which are so very important from economic point of view, since viruses are capable of inducing morphological changes in the whole plant or plant parts affecting the metabolism of the host. However, the book does not aim at to produce a reference work which can be used for diagnosing specific viral infection but within few chapters, the existing confusion resulting from the voluminous literature on the symptomatology of plant viral infection.

In this connection the author, in consultation with other workers has listed in the last chapter (Index of names of symptoms in five languages the appropriate terms for each syndrome, which will prove to be of great value to workers in different countries. The book is profusely illustrated with excellent photographs and two colour plates and it should prove to be a valuable tool for teachers, students and research workers.

——— S. P. RAYCHAUDHURI

Plant virology. Edited by M. K. Corbett and H. D. Sisler; University of Florida Press, Gainesville, Florida; 1964. 527 pp. Price $ 12.50.

With the publication of this advanced treatise on plant virology the subject touches a new height of progress which had its beginning near the end of the nineteenth century. Many review articles appeared from time to time in scientific journals, yet there was a great dearth of good text books on plant virology which to present date encompasses many supporting disciplines as well. The present status and the progress through few decades is evident from the writings of twenty two learned contributors to this volume covering many fields of virology. Further, Drs. Corbett and Sisler deserve special mention for excellent editing of the work because of which the standard of presentation has been maintained throughout at a high level. The subject matter dealt in the book covers almost all aspects of plant virology *viz.* symtomatology, vector-relationship, chemical nature, electron microscopy, structural biochemistry, infection processes etc.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The book is profusely illustrated with excellent figures and plates and at the end bibliographical and subject index have been provided. The other topics allied to the subject *viz.*, diseases of arthropods and tumour viruses etc., have also been included in the appendices. All the articles are of high standard one expects from the noted specialists in their respective fields and this latest venture, which meets a long-felt need, will be very warmly welcomed by students, teachers and researchers specially in field of virology.

S. P. RAYCHAUDHURI

Indian Science Abstracts, Vol. 1, no. 1, January, 1965; Periodicity : Monthly ; Annual Subscription: Rs. 50.00 (inland), $30.00 or £ 10 (foreign); Publisher : Indian National Scientific Documentation Centre, Delhi-12.

It is a great pleasure to welcome the first issue of a new abstracting journal—*Indian Science Abstracts*. In *Indian Phytopathology*, Vol. 17 (no. 3). 1964 a notice regarding the launching of this publication by INSDOC was inserted. Photo-offset printed, in size it approaches well known international abstracting journals like *Biological Abstracts* and others.

An *Introduction* giving the scope of the journal and historical background of abstracting service in India is followed by a list of periodicals from which papers have been abstracted. Abstracts of papers published in 99 Indian periodicals, 21 papers published by Indian workers in foreign journals, 221 patents, 28 standards, 4 annual reports, 1 thesis, 18 conference papers and 4 miscellaneous publications have been compressed in the first issue in approximately one hundred pages.

Readers wishing to consult this journal for notice about papers of phytopathological interest may do so by checking under headings such as *Microbiology* (under which papers on Plant Virology have been abstracted), *Fungi* (under BOTANY) , *Plant Diseases*, *Pests* and *Plant Protection*, *Pest Control* and under respective crop headings (under AGRICULTURE). In the end an author index and a keyword index ("based on title only") have been appended.

The reviewer feels that this type of classification of subject headings is not suitable for a quick perusal or check up. Headings such as *Mycology*, *Phytopathology* and *Entomology* would be more preferable than *Fungi*, *Plant Disease*, *Pests* etc.

If the first issue can be taken as any guide, then *Indian Science Abstracts* bids fair to become a mirror of Science in India reflecting developments in all branches albeit in an abstract fashion.

M. M. PAYAK

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Eighteenth Annual Report of the Indian Phytopathological Society for the year 1964.

Mr. President, members of the I. P. S., ladies and gentlemen,

I am presenting herewith the 18th Annual Report of the Society for the year 1964. Sixty nine new members joined the Society, 3 as life-members and 66 as ordinary members. Eight members discontinued due to nonpayment of the subscription during the year. It is a matter of great regret that four distinguished members of the Society expired during the period under report. The total number of the members at the end of the year was 369 and that of subscribers 222. Subscription is due from only a few members now.

During the year, volume XVI (4), 1963; XVII, Nos. 1,2 and 3, 1964 of Indian Phytopathology have been issued. The financial position of the Society continued to be satisfactory. Besides, a sum of Rs. 7,500/- continues to remain in the form of National Savings Certificates. The amount to the credit of the Society in the Saving Bank Account with M/s National and Grindlays Bank Ltd. (Llyods Branch), New Delhi and cash in hand taken together on 31-11-1964 was Rs. 16,650.63 Thus the assets of the Society amounts to Rs. 24,150.63. In addition, several cheques are in the process of collection.

During the year the receipt amounts to Rs. 24,420.30 and expenditure to Rs. 22,248.02, thus showing a balance of Rs. 2,172.28 in favour of the Society. It may be mentioned that no financial assistance was received by the Society from any source during the year. We have paid the cost of printing vol. XVI, Nos. 1, 2, 3 and 4 1963 and vol. XVII, No. 1 and 2, 1964 and also of symposium volume of (Bulletin No. 1) 1963. The printing of the journal has been in the hands of M/s Kapoor Art Press, New Delhi.

Three council meetings were held during the year.

I am glad to inform you that, so far 9 foreign members and 46 members in India have expressed their desire to become fellows of the Society. They will be introduced in the morning session after coffee. Also, three eminent scientists have been elected Honorary Fellows of the Society.

During the year under report the following lectures were delivered by eminent scientists :

1. 24-1-1964. MR. F. G. W. JONES, Rothamsted Experiment Station, England—"Golden Nematodes".
2. 7-2-1964. DR. J. B. GOODEY, Rothamsted Experiment Station, England—"Classification of soil and fresh water nematodes".
3. 22-2-1964. DR. M. J. THIRUMALACHAR, Hindustan Antibiotics, Pimpri—"Mycological aspects of antibiotic production".
4. 24-11-1964. DR. T. JOHNSON—Cereal Research Laboratory, Murree, Pakistan.—"Cereal rusts".

We are grateful to Prof. M. J. Narasimhan, for kindly accepting our invitation to deliver the Second Mundkur Memorial Lecture inspite of his personal inconvenience and long and tiresome journey from Poona. I am very thankful to Dr. R. Prasada and Dr. D. Surayanarayana, for kindly looking after the office of Secretary—Treasurer during my absence

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHO

Receipts and Payments account for

RECEIPTS

To *Balance on 1st January, 1963 :*		
Cash in hand	1,151.13	
Balance with National and Grindlays Bank Ltd., Lloyds Branch, NewDelhi in savings Account	14,368.23	
12 years National Savings Certificates in safe custody with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi	5,000.00	20,519.36
,, Admission Fees :		550.00
,, Life Membership Fees		4,114.52
,, Fellowship Fees		740.00
,, *Subscription :*		
1954	15.00	
1955	15.00	
1956	25.00	
1957	25.00	
1958	45.00	
1959	65.00	
1960	105.00	
1961	121.69	
1962	316.00	
1963	1,559.78	
1964	79.00	
1965	25.10	2,396.57
,, Reprints		102.82
,, Symposium Receipts		12.00
,, Journal Subscription		4,933.49
,, Interest on Savings Account		468.20
		33,836.96

Sd./- **S. P. Raychaudhuri.**

Secretary-Treasurer

New Delhi :

Dated :

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

LOGICAL SOCIETY, DELHI

the year ended 31st December, 1963

PAYMENTS

By	Establishment		1,085.00
,,	Postage		843.32
,,	Printing and Stationery		6,124.01
,,	Travelling and Conveyance		55.75
,,	Bank Charges		160.46
,,	Miscellaneous Expenses		56.04
,,	Symposium and Mundkur Memorial Lecture		318.27
,,	Annual General Meeting Expenses		164.50
,,	*Balance as on 31st December, 1963* :		
	Cash in hand	1,535.39	
	Balance with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi in Savings Account	18,494.22	
	12 years National Savings Certificates in safe custody with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi	5,000.00	25,029.61
			33,836.96

Compiled from Books of Accounts
and information supplied

Aiyar & Co.

Chartered Accountants

December 23, 1964

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

from India, for nearly six months until the end of December, 1964. Also, I am grateful to Professor C. V. Subramanian and Dr. R. Prasada for finalising everything in connection with the symposium held yesterday and today's afternoon session for reading of papers, respectively, and to Dr. M. M. Payak for his ungrudging help.

On behalf of the Indian Phytopathological Society, I express our sincere thanks to the Indian Science Congress Association and the University of Calcutta, for providing with all facilities to conduct the meetings in the Science College at Calcutta.

List of Fellows (F.P.S.I.) of the Indian Phytopathological Society elected in the Annual General Meeting of the I.P.S., held at Calcutta on December 30th, 1964.

Addy, S.K.
Arya, H.C.
Bajaj, B.S.
Balakrishnan, M.S.
*Bennett, C.W.
Bose, S.K.
Capoor, S. P.
Chaudhuri, H. C.
Chona, B. L.
*Couch, J. N.
Das, C. R.
Dastur, J. F.
Desai, M. V.
*Dodge, C. W.
Doshi, J. H.
Ganguly, D.
Ghemawat, M. S.
Grewal, J. S.
Gupta, S. C.
*Holmes, F. O.
*Horsfall, J. G.
Iyengar, M. R. S.
*Joerstad, I.
Joshi, L. M.
Kaul, T. N.
Khan, Abrar M.
Khanna, K. L.
*Lilly, V. G.
Mathur, R. S.
Mehrotra, B. S.
Misra, D. P.
Munjal, R. L.
Nariani, T. K.
Nema, K. G.
Patel, L. M.
Patel, M. K.
Pattabhiramon, T. V.
Pavgi, M. S.
Prasada, R.
Rangaswami, G.
Ravindra Nath, V.
Raychaudhuri, S. P.
Saksena, H. K.
Singh, B.
Sinha, S.
Sitalakshmi, V.
*Solheim, W. G.
Sreeramulu, T.
Srivastava, S. N. S.
*Stakman, E.C.
Subramanian, T. V.
Tandon, R. N.
Thirumalachar, M. J.
Uppal, B.N.
Vasudeva, R.S.

*Foreign, Members.

Sd/- S. P. Raychaudhuri
Secretary-Treasurer

Dated : December 30, 1964.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MANUSCRIPTS FOR INDIAN PHYTOPATHOLOGY

Contributors are requested to follow the instructions given here while preparing their manuscripts.

MANUSCRIPT COPY : Manuscripts should be typed preferably on white sheets (size $8\frac{1}{2} \times 11$ inches) on one side of the page only with at least $1\frac{1}{4}$ inch wide margin on the left and $\frac{1}{2}$ inch on each of the remaining sides. All sheets in the copy should be of one size only. Even in cases where only a few lines are to be added, full size sheets alone should be used.

The entire manuscript should be clearly typed double-spaced including title, foot notes, quotations, references and legends. The writing style should be simple and direct. Check up the copy for spellings, punctuations, diacretic marks, pagination, etc. All calculations, tables, figures, names, quotations, citations, etc. should be carefully verified before submission. Statistical data likewise must be approved by some one competent in Statistics.

Since tabular matter is expensive to print, it should be reduced to a bare minimum just necessary for substantiating the results. All tables, when included, should be consecutively numbered, and each table should have a heading stating concisely its contents.

The foot notes, if any, should be given in the same page in which their reference appears.

Instructions, if any, either to the Editor or Printer, should not be incorporated in the actual MS copy. These may be given on a separate unnumbered sheet.

The contents of the MSS should have brief headings and should broadly be given in the sequence thus : Title, (in capitals), name or names of authors, Introduction, Material and Methods, Results, Discussion, Summary, Acknowledgements, if any, Institutional Address (on left side of the page arranged flush with the margin), References and Legends for figures.

To comply with the International Rules of Botanical Nomenclature (article 36) now in force, Latin diagnosis must be supplied with each new taxon proposed. It may be emphasised that this need not be a verbatim or literal translation of the full English description. An abbreviated Latin diagnosis giving essential characters only with a full description in English will be preferred. Unnecessary duplication (such as inclusion of collection data) in both the languages should be avoided.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ILLUSTRATIONS : The available space for text figures in *Indian Phytopathology* is 4. inches in width and $6\frac{1}{2}$ inches in length. These therefore should be $4 \times 6\frac{1}{2}$ inches when reduced. As many of the figures as possible should be **grouped** and they should invariably be mounted close together leaving **no space in between** for reproduction as a single block. Mounting should be done on stiff white cardboard. Use rubber solution for pasting the figures on the board, as it is not messy.

Illustrations should be numbered consecutively in the order in which they are to appear in the text. The numerical sequence should be maintained even in cases where both photographs and India Ink drawings are included. Thus there should be only one figure bearing any particular number.

For numbering avoid use of typewritten symbols, letters, etc. Instead it can be accomplished through a lettering device (a stencil, for example) or printed letters of numbers may be used for this purpose.

Because of high cost of half tone reproduction, (used mainly for photographs, photomicrograph, etc.), carefully made line drawings in India Ink will be preferred. One page of half tone illustration is permissible per paper or article or its equivalent.

The photographs and line drawings, graphs, charts etc. should not be mounted or grouped together. The former are reproduced as half tone blocks while for the latter zinc-etching process of reproduction is used.

The drawings, done in jet black undiluted India Ink, should be bold enough for half or more reduction. Fine stippling consisting of small dots or dashes may be avoided as it often disappears in reproduction.

A scale may be added with each drawing or a group of drawings drawn at the same magnification. This is helpful for the printer to reduce the original figures to any desired size.

Original drawings will be preferred over their photostat copies since in the latter during reproduction, the background becomes murky and grey.

All unnecessary background and foreground in photographs should be trimmed. The trimming should be so done that the margins become rectilinear. Trimming which results in having grotesque or crooked margins should be avoided. Unless the edges are straight, the background has to be routed resulting in increased cost. Each figure must have a legend. The legends should be typed in double space on a separate page or pages. These should not be pasted or attached to the figures. It may be noted that the legends go to the printer while the figures are sent to the engraver.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES : All references should be double-spaced throughout. These should be listed in the alphabetical order of the authors' names after the Institutional address at the end of the paper. These may be cited as foot notes when they are four or fewer in number. In the text the references should be referred by date thus : Chaudhury (1949) or (Chaudhury. 1949). A citation should give the name of the author (or authors), his/her (or their) initials, year of publication within parentheses, and then the full title correctly, followed by the name of journal (to be underlined), number of the volume, a colon and page numbers. The diacretic signs and capitalization either in the title or in the name of the journal should be precisely as in the original.

The name of the journals should be abbreviated as given in the 3rd Edition (1952) of the WORLD LIST OF SCIENTIFIC PERIODICALS. The Biological Council of England has issued : A LIST OF ABBREVIATIONS OF THE TITLES OF BIOLOGICAL JOURNALS. Authors not having access to the WORLD LIST may consult this before finally typing the references for their papers.

If an article has not been seen in the original, then that fact should be clearly stated. When a paper in the name of two or more authors is to be cited, only the initials of the first author are to come after the surname, thus : Mueller, E. and S. K. Bose and not Mueller, E. and Bose, S.K. While citing two or more references from the same Journals and/or of the same authors avoid using words : *Ibid.*, *Loc. cit.*, *etc.* and give the full citation.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological News

CONGRATULATIONS to Director B. P. Pal of Indian Agricultural Research Institute, on his new appointment as the first Director-General, Indian Council of Agricultural Research, Government of India.

PHYTOPATHOLOGIST B. L. Chona, former Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, retired on February 13, 1965 after being associated with the Division for nearly 28 years. He proceeded to Ghana to take up a new assignment as Chief Research Officer, Ghana Academy of Sciences, Kumsai, Ghana.

CONGRATULATIONS to Prof. T. S. Sadasivan, for receiving Shanti Swarup Memorial Award for 1964.

RETIREMENT. Virus Entomologist P. M. Verma, retired from the service in November 1964 and S. N. Chatterjee was appointed in his place as Virus Entomologist at Poona.

CONGRATULATIONS to S. P. Raychaudhuri for his election to the office of the President, Section of Agricultural Sciences of Indian Science Congress to be held at Chandigarh in January, 1966. I. P. S. also congratulates him for his appointment as Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute on retirement of Dr. B. L. Chona.

PHYTOPATHOLOGISTS S. P. Raychaudhuri and D. N. Srivastava returned to Indian Agricultural Research Institute after nearly six months visit to Important laboratories in the Orient, U. S. A., U. K. and Europe on a Rockefeller Foundation Travel Programme.

MYCOLOGIST C. V. Subramanian left for C. M. I (U. K.) for a short visit on May 2, 1965.

PLANT PATHOLOGIST P. R. Mehta, Deputy Director, Plant Protection and Quarantine, Government of India, retired on August 17, 1964 and joined as General Secretary, Pesticides Association of India, New Delhi.

CONGRATULATIONS to D. Suryanarayana on his new assignment as Professor of Botany and Plant Pathology in the Punjab Agricultural University Hissar Campus.

LECTURES. Prof. John Paul of Glassgow University of California addressed the members of Society in Delhi. Prof. Krasilnikov Nicolai of U. S. S. R. also addressed the Annual Meeting held at Calcutta on December 29, 1964.

DEATHS. K. M. Thomas, former Plant Pathologist to Madras States; Mycologist L. S. Subramaniam ; Plant Pathologist K. F. Keshwalla and Phytopathologist D. I. Mathrani of Tobacco Institute (Rajmundhry).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

To evaluate methods of applying 1, 2-di-bromo-3 Chloropropane for the control of nematode root-galls—*R. B. S. Birat* 82

A virulent biotype of physiologic race 107 of brown rust of wheat: Puccinia recondita Rob ex. Desm—*R. Prasada, S. K. Sharma and Madan Mohan* 84

A new leaf-spot disease of Singhara (Trapa bispinosa) caused by Bipolaris tetramera—*G. P. Singh and S. Lal* 85

"Purple leaf" disease of beet—*S. P. Capoor and D. C. Sharma* 88

Two Powdery Mildews from Sikkim—*J. N. Kapoor* ... 90

An undescribed Myxomycete from the coffee estates of Mysore—*V. Agnihothrudu* 92

Relative efficacy of some new fungicides for the control of hill bunt of Wheat—*J. S. Grewal, P. C. Joshi, K. D. Pathak and S. B. Mathur* 94

Deodar fruit extract—an inhibitor of chilli mosaic virus—*S. P. Raychaudhuri and K. C. Chadha* 96A

Reviews 97

Eighteenth Annual Report of the Indian Phytopathological Society for the year 1964. 99

Manuscripts for Indian Phytopathology 103

Phytopathological News 106

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	36.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1965

President	M. J. THIRUMALACHAR
Immediate Past President	S. SINHA
Vice-President	K. S. THIND
Secretary-Treasurer	S. P. RAYCHAUDHURI (1965-'67)
Joint Secretary...	M. M. PAYAK (1965-67)

COUNCILLORS

Northern Zone	T. K. NARIANI
Mid-Eastern Zone	B. M. GUPTA
Eastern Zone	S. N. BANNERJEE
Central Zone	B. L. MATHUR
Western Zone	S. P. CAPOOR
Southern Zone	G. RANGASWAMI

EDITORIAL BOARD FOR 1965—'67

R. PRASADA
Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU
L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

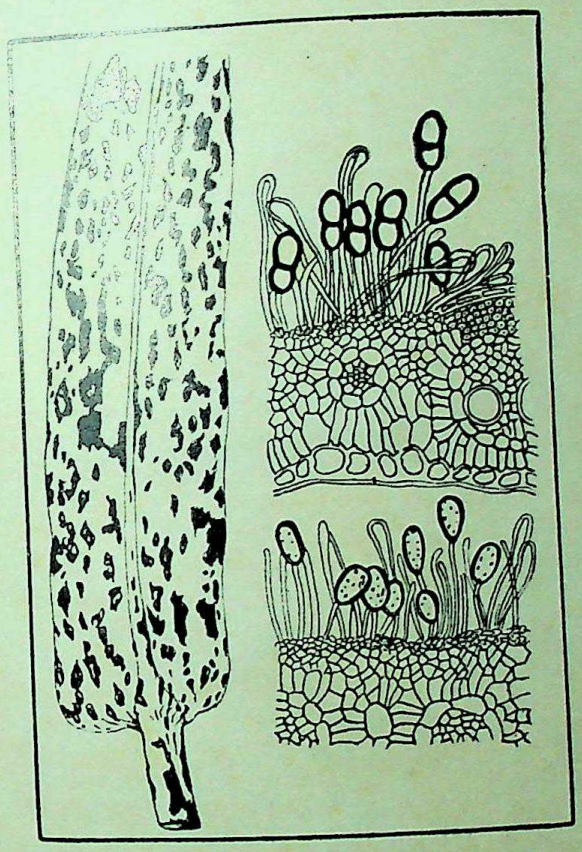
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVIII 1965 NUMBER 2



PUBLISHED FOR
THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Raghunath Prasad Asthana, 1906—1965 i

Study of heteroecious life-cycles of rusts in India—*M. J. Narasimhan* 107

Nutritional requirement of Gloeosporium musaram—*A. P. Misra and D. P. Thakur* 116

Seed Microflora II. Seed-borne fungi of Setaria italica, their distribution and control—*J. S. Grewal and Mahendra Pal* ... 123

Tests with different varieties of sesamum for resistance to Phytophthora blight of sesamum—*P. D. Gemawat and N. Prasad* 128

Mutation of Colletotrichum falcatum the causal organism of red rot of sugarcane V. Pathogenicity of biochemical mutants—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* ... 133

Studies of mosaic disease of Eleusine in the Deccan—*D. G. Rao, P. M. Varma and S. P. Capoor* 139

Studies on the uptake and translocation of labelled fungicides in plants—II. TRANSLOCATION ON FOLIAR APPLIED S^{35} IN POTATO—*Dharam Vir and B. S. Bajaj* 151

Utilization of amino acids as sole source of carbon and nitrogen by some Xanthomonads—*W. V. Kotasthane, A. C. Padhya and M. K. Patel* 154

Factors affecting conidial production of Helminthosporium oryzae—*S. B. Chattopadhyay and C. Das Gupta* ... 160

Studies on the pathogenicity and host-range of three species of Root-knot nematodes—*A. Chidambaranathan and G. Rangaswami* 168

Evaluation of host specificity character in the differentiation of Xanthomonas species—*M. G. Patel, M. C. Srinivasan and M. J. Thirumalachar* 174

Toxicity, physical properties and biological spectrum of certain copper and organic fungicides—*A. P. Misra and T. B. Singh* 181

Studies on sugarcane rust in India—*M. L. Sahni and B. L. Chona* 191

(Contd on. 3rd cover)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RAGHUNATH PRASAD ASTHANA, 1906-1965

Dr. Raghunath Prasad Asthana

In the death of Dr. Raghunath Prasad Asthana at Allahabad on July 24, 1965, India has lost an eminent plant pathologist and a renowned teacher.

Born at Gorakhpur (U. P.) on February 20, 1906, Asthana received his early education at various places in U. P.; obtained M. Sc. degree in Botany from the University of Allahabad in 1930 and Ph. D. and D. I. C. from the University of London in 1932.

On his return from London, Asthana joined the Department of Botany, Allahabad University, for a short period and left in 1935 to take up an appointment in the Agra College as Assistant Professor of Botany. From 1941-46 he was Mycologist to Government of Central Provinces and Professor of Plant Pathology, College of Agriculture, Nagpur, and was Principal from 1956 till his retirement from service in 1961. He was also Dean, Faculty of Agriculture, Nagpur University as also Member of its Academic and Executive Councils. After his retirement from Government Service, Asthana continued his researches in the Department of Botany, Allahabad University, with the financial assistance of Indian Council of Agricultural Research under their scheme of "Utilization of the services of retired scientists in the field of Agriculture" and at the same time he maintained his interest in teaching as Hony. Director and Professor of Plant Pathology, K. A. College of Agriculture, Allahabad.

Asthana was connected with several scientific societies. He was once a Vice-President and the Editor-in-Chief of Indian Phytopathological Society and Indian Phytopathology, respectively; Fellow of Linnean Society, Indian Botanical Society, Indian Academy of Sciences and Indian Phytopathological Society. He was a member of Plant Diseases Committee of Indian Council of Agricultural Research and Technical Advisor to State Public Service Commission, Nagpur, and to Union Public Service Commission, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Apart from the persuit of Plant Pathology as a career, Asthana had varied interests in social and cultural activities. He was Past Master, Free Masonic Lodges at Nagpur and Allahabad, and Member of Rotary International. He was a popular figure amongst the staff and students and was respected and loved for his keen interest in sports, dramatics, music and all activities concerning students' welfare.

Asthana published more than 45 scientific papers in his subject both in Indian as well as foreign journals and made a rich contribution to our knowledge of plant diseases and their control. Many of us who looked upto him for inspiration and guidance will miss him very much. His passing away has left a void in the rank of plant pathologists of India at a time when the country could ill-afford such a loss. Dr. Asthana is survived by his loving wife, two sons, one daughter and a grandson, and to them Indian Phytopathological Society extend their condolences and heartfelt sympathy in their irrepairable loss.

R. PRASADA

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDY OF HETEROECIOUS LIFE-CYCLES OF RUSTS IN INDIA*

M. J. NARASIMHAN

Director of Agriculture (Retd.), Mysore State

I feel honoured to be invited to deliver the Second Mundkur Memorial Lecture, in succession to the brilliant address given by Prof. J. F. Dastur, who delivered the first lecture. While Prof. Dastur knew Dr. Mundkur as an official colleague for several years, my acquaintance with him goes much earlier, when he was a student of the Presidency College. Madras and was several years junior to me. Even as a student he was marked by the outspoken way of presenting his ideas, which was a dominant feature all through his research career. After the student days we lost contact for several years, until about 1936 when he burst out on myself and late Dr. M. O. P. Iyengar, by publishing a note criticising the nomenclature of a paper by us on *Schizonella colemanii*, a genus of smuts which we described on the inflorescence of *Vitis quadrangularis*. His paper, though short, brought home on us all the care that has to be excercised in observing the rules of nomenclature, etc. Many a time the importance of good results of cultural studies is sacrificed in place of a pedantic description of a new genus or a species. In later years in Mysore we had several occasions to meet each other and had long discussions, and many of these have contributed to inspire me to take up studies on rust fungi, *especially on heteroecism* after my retirement from Government Service.

My first interest in the rust fungi came up nearly 40 years ago, when as junior mycologist of the Department of Agriculture, Mysore, I was confronted with the problem of the leaf rust of coffee incited by *Hemileia vastatrix* Berk. and Br. This rust as you know, is a classical disease and at that time, it was next in importance only to wheat rusts. The personality of Marshall Ward and all his detailed work was before us, and the finding of the possible alternate host for the coffee leaf rust was of primary importance. If all the negative results of studies carried out in finding the alternate host for *Hemileia vastatrix* could be published in some place, it would run to at least three numbers of *Indian Phytopathology*. The work had to be carried out in the Coffee Experimental Farm, Balehonnur, in the midst of dense jungle and heavy downpour of rain. In those days the easy access of shooting a note to one of the scientific journals was not there and hence most of the valuable data were piled up year after year, only to be preserved in the files, since many of the Directors of Agriculture in Mysore did not like certain aspects of mycological and plant pathological work. Hence most of the data published were series of papers on spraying of coffee for leaf diseases with reference to epiphytology. The looking for any aecial stage in the vicinity of coffee estate became almost a mania, and each one of them was studied with abounding interest until it was proved to

*Second Mundkur Memorial lecture delivered on 29th December, 1964.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

be a rust not connected with the life cycle of coffee leaf rust. After 40 years of work I may state that to this day the pycnial and aecial stages are unknown not only for *Hemileia vastatrix* but of the genus *Hemileia* itself. The claims about occurrence of uredinoid aecia etc. made by F. L. Stevens for *Hemileia wrightiae* are only uredia themselves.

Eventhough my earlier work was concerned with the study of sexuality of fungi specially *Phytophthora arecae*, *Corticium koleroga*, and cytological studies on the sandal spike disease including the showing for the first time its viral nature, etc., the problem of heteroecism in rust fungi has always appealed to me as a challenging subject. Its importance need not be told to you, since devising of control measures, study of origin of physiologic races, etc. are all connected with the aecial stage of the rust, though, by heterokaryosis it is often possible to trace the development of a new race on the uredial host. The year 1865 was a landmark, when the great German professor of the University of Starsbourg, Anton de Bary established the fact that the aeciospores from barberry caused infection on wheat plant. Almost hundred years have elapsed, and the brilliance of his work, the methodical way of his approach to the problem are still shining as beacons for all students of fungi. In the following years large number of eminent uredinologists have made great contributions and laid firm foundations for future workers. Among these prominent scientists mention may be made of Klebahn, Dietel and Hennings in Germany, Arthur, Kern, Mains, Jackson, Fromme, and Cummins in the United States, Naoharu and Naohide Hiratsukas in Japan, Eduard Fischer, Eugene Mayor, and Gäumann in Switzerland, Lagerheim, Ivar Joerstad, Nannfeldt, Liro and Rostrup in Scandinavia, Hariot, Patouillard and Guyot in France, Tranzschel In U.S.S.R. These are some of the most prominent contributors. To this be added the works of K.C. Mehta, Thirumalachar and Mundkur in India, McAlpine in Australia, Cunningham in New Zealand, and Faull in Arnold Arboretum. The great bankers, H. Sydow and P. Sydow, along with Trotter in later years took over the whole field of systematic studies of rust fungi, though very little culture work was done. I should not forget to mention here the fine work of Pole Evans and Miss Ethel Doidge in South Africa, who had a situation similar to that in India when they started their work and accomplished much.

The earlier investigators had already published a mass of valuable data by a series of cultural studies. Klebahn (1904) reported 150 heteroecious species belonging to 10 genera. Arthur (1921) in a series of work on cultural studies reported over 100 heteroecious rusts. These have been summed up in Dietel's paper (1918) where over 264 such heteroecious rusts are reported. Our own achievement in India appears to be very small compared to the gigantic contributions of the workers referred to above, though there appears to be no dearth for the abundance of material.

The heteroecious nature of rust is the most intriguing thing, which in a general way is comparable to the alternate or intermediary hosts seen in the case of animal parasites. For instance, in a single group like the tape worms in which several genera are included, how nature ever contrived to put the pathogen in such an advantageous position to find

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the proper host, is most baffling. For the tape worm of man, pig is the alternate host, and man eating pork gets the infection. For the tape worm of fowl, ants are the alternate host, and for the tape worm of cats, rats are the collateral hosts. Like this, the examples may be cited in any number. In the same way, the alternate hosts for the rusts are most interesting. *Rhamnus* species were usually used in Europe as fence for oat fields, and the aecial stage of *Puccinia coronata* on oat occurs on *Rhamnus*. In most of the cases the two hosts occur in close juxtaposition.

As regards the origin of heteroecious and autoecious rusts, there have been large number of concepts developed in the last several years. Eduard Fischer (1898) and several others thought that heteroecious rusts are primitive and the autoecious ones are derived from them. Klebahn (1904) and others held the view that heteroecious rusts arose from autoecious rusts. The detailed paper by Jackson (1931) has cleared the concept that the heteroecious rusts are the older ones and the autoecious rusts are derived from them. By short-cycling and similar tendencies, the eu-forms gave rise to opsis,brachy-forms and finally to the microforms. Some of the aberrant rusts like *Puccinia minussensis* studied by Buller (1942) and others have provided excellent examples for these concepts.

Considering the large number of rust genera and species that occur in India, the paucity of cultural studies on rusts is striking. It is true that such type of work often does not result in large number of publications as in other branches of mycology, particularly systematic mycology. But even a small piece of good cultural study should be rewarding if it is successfully carried out. The pioneer worker in the field of heteroecious rusts in India was Major Alfred Barclay. Like some of the other scientifically minded army officers sent by the British Government to India, Barclay carried out admirable mycological work. He made extensive collections of rusts and smuts, some of which he sent to Oscar Brefeld in Germany. One of the smuts *Neovossia barclayana* was named by Brefeld in honour of this indefatigable collector. He studied the life-cycle of *Uromyces hobsoni* and the illustrations of the detailed work he has published in the *Transactions of Linnean Society* would be an example for many of us to copy and learn. He established the genus *Monosporidium* which was mishandled taxonomically by various workers in recent years, and has been put in its right place as a valid genus by Thirumalachar. His most outstanding contributions however are the studies on heteroecious rusts about which I would give a brief account. To this day in India, no other worker on cultural studies of rusts has surpassed Barclay either in the volume of work or its brilliant exposition. If in addition to his work as an army officer he found time to achieve so much, I do not understand why with equal devotion to work and good planning, it should not be possible for some of us to emulate him.

Barclay (1887) studied the edible rust which forms the hypertrophied aecial stage on *Urtica parviflora*, and connected it with the telial stage of *Puccinia carexis* var. *himalayensis*. *Puccinia polliniae* was connected with the aecial stage on *Strobilanthus dalhousianus* (1887). The crown

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

rust *Puccinia coronata* was shown to have the aecial stage on *Rhamnus purpurea* and *R. procumbens* in the Himalayas. It developed telia on *Stipa* and *Poa* grasses also (1891). The grass rust *Puccinia chrysopogonis* was shown to be connected genetically with the aecial stage on *Jasminum humile*. Barclay (1890) also studied the heteroecious nature of *Gymnosporangium cunninghamianus* and found the telial galls on *Cupressus torulosa*. The aecial stage was on *Pyrus pashia*. These and other brilliant contributions have earned him a unique place in the history of Indian Mycology. I may mention here my feeling of surprise that Barclay's name does not appear in the comprehensive publication of Dr. Maheshwari on *Fifty Years of Botany in India*. This may be excused since Dr. Maheshwari is a world renowned plant morphologist, and some of the soft corners of a mycologist would not be felt by him in the same way.

The next series of works to be mentioned are those of Bagchee, (1941) who working on the rust of *Pinus longifolia* connected it with a *Cronartium* stage on *Swertia* species. The rust was named *Cronartium himalayense*. Following this fine work he successfully connected an aecial stage on *Pinus excelsa* with the uredial and telial stages on *Senecio rufinervis* and *S. alatus*. The rust was named in honour of Major Barclay with the name *Coleosporium barclayense* (1950). Ramakrishnan and Soumini (1948) showed that the bajra rust *Puccinia penniseti* has aecial stage on the egg plant *Solanum melongena*. Ramakrishnan and Sundaram by cultural studies showed that the rust *Puccinia romagnoliana* on nut grass *Cyperus rotundus* has aecial stages on Convolvulaceae and that *Puccinia rufipes* has aecial stage on *Thunbergia alata*. Hardev Singh reported that *Puccinia aristidicola* develops aecial stages on *Pupalia* species. Patil and Thirumalachar (1964) had earlier established the same connection but failed to get early publication. Prasada (1946, 1947) experimentally established the connection of aecidia commonly found on *Thalictrium* and *Berberis* in Simla Hills with the brown and black rusts of *Agropyron*, respectively. Joshi and Payak (1963) showed the aecial stage of *Puccinia poae-nemaralis* occurs on *Berberis*. All these are very important contributions; though sporadic they are highly praiseworthy.

An aecial stage on *Polygonum* species was reported to have connection with the uredial and telial stages on *Phragmitis commune* by Sanwal in Delhi. He carried out cross-inoculation studies, and identified the rust as *Puccinia invenusta* Syd. for which no aecial stages were known before. A careful perusal of his work showed that the rust he was dealing with was *Puccinia phragmitis* (Schum.) Koern, which is reported to occur on species of *Phragmites* in India. This rust has been studied in great detail by several investigators, particularly Parmelee in Canada. It has alternate host on *Polygonum Rheum* and *Rumex* species, and what has been reported by Sanwal on *Polygonum* species in India is the same rust.

I started work on the heteroecious rusts on cereals and grasses in 1941. We had already established the heteroecious connection between *Dastudinella divina*, the giant bamboo rust with the aecial stage on the Rubiaceous host *Randia dumetorum*. This was the first heteroecious rust which gave us all the necessary experience to formulate techniques for further studies. As recently as 1963, Cummins reported life-cycles of several of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the rusts based upon mere field associations. For instance he would notice aecial stages on a particular host, and after a few months in the same place he would collect the uredial stage on another host and make suggestion that they are possibly connected. For instance, he noted *Puccinia aristidae* on *Aristida* species under *Boerhaavia* with aecia and concluded the connection thereof. Because it comes from an eminent authority like Dr. Cummins, very few people doubt it and the concept gets established in future literature. I do not think it is correct to accept such field observations as proving anything, unless these data are established by cultural studies based on cross-inoculation experiments. If we merely accept field associations as having any value, they are only pointers for taking up further work and one should not conclude anything. At that rate, each one of us would be giving an aecial host for *Puccinia striiformis* on wheat and *Hemileia vastatrix*.

The work on *Puccinia versicolor* parasitising the leaves of *Heteropogon contortus* was completed following the discovery of aecial stage by myself and Thirumalachar (1950) on *Canthium parviflorum*, a member of the Rubiaceae. For discovering the alternate host, the following assumptions were made, which in the course of last several years have paid off rich dividends. These were the following :

(1) The first outbreak of the uredial stage of a rust is usually in the vicinity of its alternate host, if the rust is heteroecious. After the initial development of the uredial stage, the spread of secondary infection is so rapid and erratic in distribution, that it is difficult to trace the first place of origin of the source of inoculum. The epiphytology work on wheat rust has amply shown this, and it would be very difficult to draw any conclusions.

(2) A good survey of the aecial stages on a particular host, and the subsequent development of the uredial and telial stages on various other hosts must be carefully recorded. Of course, the investigator must know that the aecial stage of the rust does not belong to an autoecious cycle or an *Endophyllum*, in which case it would only result in waste of efforts. By analysing the data he would soon find out that only one or two uredial stage bearing hosts would consistently be associated in every place where the aecial stage occurs. For instance, in the heteroecious rust *Puccinia cacao* (1954) which we studied, the systemic aecial stage occurred on *Hygrophila spinosa*, which was described earlier by Arthur and Cummins from the Philippines under the name *Aecidium manilense*. In Patna where we, made the survey for discovering the uredial host, we found nearly 15 uredial hosts in Patna city area, and five uredial hosts near Bihar Sheriff. Near Phulwari Sheriff, there were only two uredial hosts, one of them was *Rottboelia compressa* and the other, *Cyperus rotundus* with *Puccinia romagnoliana*. By inoculating the aeciospores from *Hygrophila spinosa*, uredial infections were produced only on *Rottboelia compressa*, thus establishing connection between *Aecidium manilense* and *Puccinia rottboeliae*. Sporidial inoculations confirmed the genetic connection. Even so, it would be most rewarding if a co-operative work is carried out in India for discovering the aecial stage for the sorghum rust, *Puccinia purpurea*, which must surely

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

occur somewhere in India. Mycologists can carry out co-ordinated field studies and track the aecial host. Some complacency was brought to this problem by the reporting of Dickson *et al.*, that the rusts, *Puccinia sorghi*, *P. polysora* and *P. purpurea* form a complex group, having *Oxalis corniculata* as the alternate host. While it is true that *Oxalis* serves as alternate host for *P. sorghi*, its connection with *P. purpurea* is incorrect. In one of the correspondence with Dr. Dickson, he did not rule out the possibility of there having mixed-teliospore inoculum in the work with the aecial stage for *P. purpurea*.

Our work (1964) on the heteroecism of the rust on *Setaria italica* was most interesting. *Uromyces setariae-italicae* Yoshino occurs on a large number of hosts. Since many of the *Setaria*, *Brachiaria*, *Panicum* species on which also the same rust occurs in abundance, harbour the uredial stages during summer months also, it was easy to visualise a case of oversummering of the rust in the uredial stage itself. Cross-inoculation experiments of the uredial stages revealed that the uredial stages were host specific. In the meanwhile, the occurrence of heavy infection of *Aecidium brasiliense* on *Cordia rothii* in the vicinity of Poona was noted. This rust was first reported for India by Professor S. L. Ajrekar, and the rust has wide distribution in the world. Following the techniques previously stated, the rust was traced to *Setaria italica* and *Panicum repens*. Finally the aecial stage was connected with the rust on *Setaria italica*. The germination of the teliospores was of the unique type, by the development of a large vesicle, from which the promycelium protruded out as a side branch.

Uromyces clignyii is a rust widely distributed in South Africa, parts of Europe and Central America. It was first described by Hariot and Patouillard on a species of *Andropogon*. The uredial stages and covered telia bearing one-celled spores are characteristic. The rust occurs in India on a wide range, having been variously mis-named. Sydow and Butler called it *Uromyces andropogoni-annulati* and Ramakrishnan *et al.*, described *Uromyces triandrae* on *Themeda wellingtonica*. As Cummins has rightly pointed out, all these species have to be referred to *Uromyces clygnii*. The rust occurs in abundance in Maharashtra. Soon after the rains, an aecial stage on *Chlorophytum* was noticed in several localities. A detailed survey and careful analysis according to the methods already outlined, suggested that the two may be connected. The aecial stage was produced by sporidial inoculation of the leaves of *Chlorophytum* and similarly from the aeciospores of *Chlorophytum*, the uredial and telial stages were produced on *Heteropogon contortus*. Literature indicated that the aecial stage is identical with *Aecidium hartwigii* described by Kalchbrenner from South Africa, and in the same locality, *Uromyces clygnii* is also reported. Hence it is clear, the same alternation of generations exists in other countries also.

The most exciting experience has been with *Puccinia d[illegible]*, a rust known on *Dichanthium annulatum*, reported from several countries wherever the plant is known to occur. The rust is ubiquitous, and it is hard to miss it in the field. If one had started from the telial stages of the rust and tried to find out the aecial stage, one would end up in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

inoculating a host of genera and species of plants. Fortunately, we started from the other end. Having worked on the systemic rust on *Hygrophila spinosa*, we became interested in a similar rust on *Barleria speciosa*, previously named *Aecidium barleriae Aesalamii*, etc. This rust incites systemic infection and has many characters in common with the aecial stage on *Hygrophila spinosa*. During certain temperature conditions, it produces only sterile pycnidia and under favourable conditions, pycnia with flexuous hyphae followed by aecial development is seen, as in *Puccinia cacao* on *Hygrophila spinosa*. When the fertile aeciospores were collected and their function studied, the occurrence of the uredial and telial host was traced, and this was shown to be *Puccinia duthiae*. Following this, large-scale inoculation and cross-inoculation experiments were carried out and heteroecious nature was confirmed. As in the case of *Puccinia cacao*, the dormant systemic mycelium on the aecial host oversummered, and new shoots developing from these always produced abundant pycnia and aecia. I may continue with more instances of heteroecious rusts which we have connected, but I will take another opportunity to present these data.

When an alternate host for a rust has been successfully established, it is usual for us to assume that the final results have been achieved. Arthur in his early work found that *Puccinia subnitens* on *Distichlis* had its aecial stage on *Chenopodium album*. His further work showed that the aecial stage of this rust occurs on 76 different hosts belonging to 19 families. McDonald found that *Uromyces lineolatus* on *Scirpus* species had aecia on nine members of the Umbelliferae. *Puccinia aristidicola* has numerous hosts, and the one on *Pupalia amentacea* added up from Indian work is an additional one. Patil and Thirumalachar (1964) added up *Lantana indica* as an additional aecial host for *Puccinia versicolor*, which previously was reported only on *Canthium parviflorum*. These studies are important because, for some of the rusts of economic importance, our ideas from foreign work are so strongly entrenched, that we fail to recognise any other alternate host even if it exists.

I have already pointed out the various theories concerning the origin of heteroecism in rusts. The Tranzchel's law, based upon the fact, that in short-cycling, the telial stage is formed in the course of evolution, on the aecial host gives the following clue. If a host bears an aecial stage, and an unconnected telial stage which in morphology resembles closely another rust on a different host, it is possible, that the unconnected aecial stage is genetically connected with the telial stage on the said host. Cummins (1936) studying the arrangement of the pores on urediospores formulated the hypothesis that the scattered arrangement of pores is possessed more frequently by heteroecious species than by autoecious species, which have equatorial pores. In the heteroecious Melampsoraceae none have equatorial pores, with exceptions such as *Hyalopsora*, and *Bubakia*. However it be, it is better to rely on cultural work than on statistical inferences. I will point out here another aspect of the significance of the heteroecious nature of the rusts. As already stated, on a particular host the first outbreak of the uredial stage of a rust is in the vicinity of its aecial host. Where the aecial and uredial hosts occur naturally, it is the epicentre for infection. In cultivated plants

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

which are transported from place to place, and from continent to continent, often, the aecial hosts are also inadvertantly transported, as in the case of barberry which was taken to America for its berries. In such cases, new epicentres are formed as a result of activity of man. When one takes a crop like coffee, there is no point in looking for the aecial host of *Hemileia vastatrix* either in India or Ceylon. Search must be made in the mountains of Ethiopia where the coffee was first known to occur naturally, and the rust also develops. In case of *Hemileia vastatrix*, I firmly believe, that the aecial stage, if it occurs, would be in the mountains of Ethiopia. The plant rusts being obligate parasites, must occur along with their hosts. Where a particular rust is restricted to a particular locality and both the telial and aecial hosts occur in the same place, it may be surmised, that the particular telial host is a native of that place and not introduced either by natural or artificial means. This surmise is important because, one of the hosts may be introduced, but the possibility of both the hosts being introduced, into the same locality is improbable. This may not apply for many of the other rusts which are widely distributed, and the uredial and aecial hosts also occur in abundance. In a limited way the heteroecious conditions would throw light on the history of origin and distribution of the host plants also.

In this address, I have attempted to bring out the importance of work on heteroecism of rusts in India, which Dr. Mundkur wished to take up nearly forty years ago, but never could or did. There are about 76 species of the form genus *Aecidium* mentioned in the Fungi of India by Butler and Bisby, and many more not recorded therein. I wish young Mycologists in our country to take up cultural work, and not merely rest content with naming of species.

References

ARTHUR, J. C. *Mycologia*, **13** : 12-24, (1921)

BAGCHEE, K. D. *Indian Forest Rec.* (*Bot. Sec.*), **18** : 11-66, (1933)

——— *Indian Forest Rec.* (*N.S. Bot.*), **4** : 43-64, (1950)

BARCLAY, A. *Scient. Mem. Med. Officers Army of India*, **2** : 15-27, (1887)

——— *Scient. Mem. Med. Officers Army of India*, **2** : 29-38, (1887)

——— *Scient. Mem. Med. Officers Army of India*, **5** : 79-86, (1890)

——— *Trans. Linn. Soc. London*, (*II. Ser. Bot.*) **3** : 227-236. (1891)

——— *Trans. Linn. Soc. London*, (*II. Ser. Bot.*), **3** : 237-242, (1891)

BULLER, A. H. R. *Botanical Review*, **7** : 335-431, (1942)

de BARY, ANTON, *Monastber. K. Preuss Acad. Berlin*, (1865).

CUMMINS, G. B. *Mycologia*, **28** : 103-132, (1936)

CUMMINS, G. B. *Mycologia*, **55** : 73-78, (1963)

DICKSON, J. G. *Diseases of Field Crops*, (2nd edit.) by McGraw Hill Publication.

DIETEL, P. *Centarbl. Bakt. II.* **48** : 470-500, (1918)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

1965]

FISCHER, Ed. *Betr. Krypt. Schweiz.*, **1** : 1-120, (1898)

JACKSON, H. S. *Bull. Torrey Bot. Cl. Memoirs* (1931).

JOSHI, L. M. AND M. M. PAYAK. *Mycologia*, **55** : 247-250, (1963)

KLEBAHN, H. *Die wirtwechselenden Rost Pilze*, (1904)

NARASIMHAN, M. J. AND M. J. THIRUMALACHAR. *Mycologia*, **46** : 222-228, (1954)

———— *Mycologia*, **56** : 555-559, (1964)

PAMELEE, J. A. *Mycologia*, **55** : 133-141, (1963)

PRASADA, R. *Curr. Sci.*, **15** : 54-56, (1946).

————, *Indian J. agric. Sci.*, **17** : 137-151, (1947)

PATIL, B. V. AND M. J. THIRUMALACHAR. *Curr. Sci.*, **33** : 253, (1964)

RAMAKRISHNAN. T. S. AND C. K. SOUMINI. *Indian Phytopath.*, **1** : 97-103, (1948)

THIRUMALACHAR M. J. AND M. J. NARASIMHAN. *Ann. Bot., Lond., N.S.* **14** : 341-343, (1950)

———— AND————K. S. GOPALKRISHNAN. *Bot. Gaz.*, **108** : 371. (1947)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

NUTRITIONAL REQUIREMENT OF GLOEOSPORIUM MUSARUM

A. P. MISRA AND D. P. THAKUR

(Accepted for publication April 10, 1965)

INTRODUCTION : Tandon and Grewal (1956) and Grewal (1957) reported the selectivity of *Gloeosporium musarum* Cke. and Massee for carbon and nitrogen uptake. Mann (1944) and Agarwal (1958) showed the indispensability of sulphur and phosphorus for the growth of *Aspergillus niger* and *Curvularia penniseti* but no such studies seem to have been made with *G. musarum*. In the present investigations the carbon, nitrogen, sulphur and phosphorus requirements for growth and sporulation of the fungus were studied in details.

MATERIAL AND METHODS : The fungus was isolated from naturally infected banana fruit from Sabour (Bihar). To study the growth charal cters of the organism Richard's synthetic medium (Sucrose 50 gr., KNO_3 5 gr., $MgSo_4$ 2.5 gr., $Fecl_3$ 0.02 gr. and distilled water 1000 ml.) was used. Different carbon sources were incorporated separately in the basa-medium devoid of any carbon source at the same carbon level as present in 50 gms. of sucrose in the standard Richard's medium. Similarly different sources of nitrogen, sulphur and phosphorus were added separately at the same levels of nitrogen, sulphur and phosphorus as present in Richard's medium. The medium was adjusted to pH 5 in each case.

Twenty-five ml. of the medium was dispensed in 100 ml. Erlenmeyer flasks, autoclaved at 15 lbs. pressure for 25 minutes and inoculated with equal amounts of inoculum from 8-10 days old PDA culture. In all cases the cultures were incubated at 30° C for 21 days, after which, the growth was determined by the dry weight of mycelial mat and extent of sporulation by Haemacytometer counts (Peiris 1947).

EXPERIMENTAL RESULTS : The fungus showed marked selectivity in utilizing different carbon sources (Table 1). Lactose proved to be the best source of carbon for maximum growth followed by arabinose, galactose, sucrose, mannitol, maltose and starch in order. Besides control (devoid of carbon) there was no growth in tartaric acid. Statistically, lactose, arabinose, galactose and sucrose were equally effective. Sporulation too, was maximum in lactose followed by starch, glucose, sucrose, maltose, mannitol, arabinose and galactose. The fungus produced the largest conidia in mannitol.

The growth of the fungus increased with the increase in concentration of carbon upto 3 percent, beyond that the growth decreased in irregular manner (Table 1). The maximum growth was recorded at 2.5 and 3.0 per cent carbon levels. Statistically too, the treatment differences were significant over control. Extent of sporulation was maximum at 2.5 per cent carbon level, while there was no consistent effect of different carbon levels on the size of conidia.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Sources and levels of carbon in relation to growth and sporulation of *G. musarum*.

Sources of carbon			Concentrations of carbon		
Sources of carbon	Dry mycelial wt. (mg.)	Spores per ml.	percentage Carbon (Lactose)	Dry mycelial wt. (mg.)	Spores per ml.
Sucrose	493.3	29×10^6	0.00	Nil	Nil
Lactose	576.7	79×10^6	0.50	121.7	9.22×10^5
Maltose	456.7	22.5×10^6	1.00	127.0	1.22×10^5
Glucose	436.6	30.5×10^6	1.50	230.0	1.70×10^5
Galactose	536.6	20.5×10^6	2.00	317.0	2.40×10^5
Arabinose	556.7	21.5×10^6	2.50	427.0	143.0×10^5
Mannitol	483.3	22×10^6	3.00	556.7	29.0×10^5
Tartaric acid	Nil	Nil	3.50	343.0	21.7×10^5
Starch	343.3	53.5×10^6	4.00	395.0	17.0×10^5
Control	Nil	Nil	4.50	358.3	1.0×10^5
C. D. for mycelial growth (P=05) 66.78			C. D. for mycelial growth (P=05) 35.91		

Peptone, urea and calcium nitrate supported the maximum growth and sporulation of the fungus (Table 2). Sporulation was good in potassium nitrate, sodium nitrate and asparagine. The fungus was capable of sporulating even without any nitrogen. The size of conidia followed the same pattern as those of growth and sporulation.

Nitrogen at 0.15 per cent level proved significantly superior to all other concentrations tried, while further increase resulted in progressive decrease of mycelial output. Extent of sporulation and size of conidia were also maximum at 0. 15 per cent nitrogen level (Table 2).

Sodium thiosulphate yielded maximum growth of the fungus followed closely by sodium sulphite and sulphates of ammonium, magnesium and zinc (Table 3). Sulphates of sodium and potassium proved to be very poor for growth, whereas neither growth nor sporulation was observed in sodium sulphite showing its inhibitory effect on the fungus. Ammonium sulphate, though gave good growth failed to produce spores. Extent of sporulation was maximum in magnesium sulphate but sodium sulphate produced the largest conidia.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Sources and levels of nitrogen in relation to growth and sporulation of *G. musarum*.

Sources of nitrogen			Concentrations of nitrogen		
Sources of nitrogen	Dry mycelial wt. (mg.)	Spores per ml,	Percent nitrogen (urea)	Dry mycelial wt. (mg.)	Spores per ml.
Sodium nitrate	456.7	28.8×10^6	0.00	256.7	7.2×10^6
Potassium nitrate	460.0	30.4×10^6	0.05	435.0	26.4×10^6
Calcium nitrate	473.3	33.6×10^6	0.10	373.3	46.4×10^6
Ammonium nitrate	406.7	7.2×10^6	0.15	530.0	65.6×10^6
Ammonium Sulphate	396.7	Nil	0.20	470.0	33.6×10^6
Ammonium chloride	376.7	Nil	0.25	430.0	23.2×10^6
Urea	510.0	76.0×10^6	0.30	426.2	16.8×10^6
Peptone	516.7	79.2×10^6	0.35	333.3	10.4×10^6
Asparagine	440.0	21.6×10^6	0.40	263.3	8.0×10^6
Control	167.0	12.0×10^6	0.45	226.7	5.6×10^6
C. D. for mycelial growth (P=05) 47.9			C. D. for mycelial growth (P=05) 50.25		

There was progressive increase in mycelial output with increase in sulphur level reaching a maximum at 0.05 per cent beyond which the growth declined (Table 3). The different treatments, though did not differ significantly among themselves, yet differed significantly over control (devoid of sulphur). Optimum levels of sulphur to yield maximum spores was 0.06 per cent while the conidia attained maximum length at 0.05 per cent sulphur level.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Sources and levels of sulphur in relation to growth and sporulation of *G. musarum*.

Sources of sulphur			Concentrations of sulphur		
Sources of sulphur	Dry mycelial wt. (mg.)	Spores per ml.	Percent Sulphur (Mag. sulphate)	Dry mycelial wt. (mg.)	Spores per ml.
Magnesium sulphate	306.7	11.90×10^4	0.00	143.3	0.48×10^6
Potassium sulphate	243.3	1.38×10^4	0.02	343.3	39.20×10^6
Ammonium sulphate	346.7	Nil	0.03	343.3	20.80×10^6
Sodium sulphite	240.0	2.70×10^4	0.04	356.7	30.50×10^6
Sodium sulphite	380.0	4.20×10^4	0.05	363.3	19.20×10^6
Sodium bisulphite	Nil	Nil	0.06	346.7	48.00×10^6
Sodium thiosulphate	440.0	4.20×10^4	0.07	356.7	26.40×10^6
Zinc sulphate	293.3	0.14×10^4	0.08	350.0	33.60×10^6
Control	250.0	0.72×10^4	0.09	343.3	23.20×10^6
			0.10	340.0	28.00×10^6
C. D. for mycelial growth (P=05) 61.8			C. D. mycelial growth (P=05) 51.47		

The fungus attained the maximum growth, sporulation and largest conidia in dibasic potassium phosphate followed by tribasic potassium, monobasic potassium and ammonium phosphates. Statistically, both dibasic and tribasic phosphates were equal. Phosphorus seemed to be an essential element as the medium devoid of phosphorus produced least growth, sporulation and smallest conidia (Table 4).

Increased levels of phosphorus (*Dibasic potassium phosphate*) had direct bearing on increased mycelial output with the maximal growth at 0.25 per cent. Beyond this, the growth reduced progressively. Statistically phosphorus from 0.2 to 0.4 per cent levels gave similar results, but

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

proved superior to the rest of the concentrations tried. Maximum sporulation was observed at 0.15 per cent phosphorus level but the conidia were smallest at this level (Table 4).

TABLE 4 : Sources and levels of phosphorus in relation to growth and sporulation of *G. musarum*.

Sources of phosphorus			Concentrations of phosphorus		
Sources of phosphorus	Dry mycelial wt. (mg.)	Spores per ml.	Percent phosphorus (Dibasic pot. phosphate)	Dry mycelial wt. (mg.)	Spores per ml.
Monobasic Potassium phosphate	293.03	1.7×10^5	0.00	156.7	1.4×10^5
Dibasic Potassium phosphate	370.00	6.3×10^5	0.05	263.3	1.7×10^5
Tribasic potassium phosphate	336.70	4.9×10^5	0.10	270.0	2.6×10^5
Ammonium phosphate	236.70	4.9×10^5	0.15	280.0	13.3×10^5
Control	196.70	0.4×10^5	0.20	303.3	7.6×10^5
			0.25	350.0	6.9×10^5
			0.30	346.7	6.6×10^5
			0.35	310.0	5.8×10^5
			0.40	303.3	1.3×10^5
			0.45	283.3	1.7×10^5
C. D. for mycelial growth (P=05) 35.74			C. D. for mycelial growth (P=05) 48.5		

DISCUSSION : Studies on the growth, sporulation and size of the conidia of *G. musarum* to different sources of nutritive elements showed the specificity of the organism to utilize those elements. The nine carbon sources used can be classified into three distinct groups. Lactose, arabinose and galactose were the most readily available carbohydrates, while sucrose, mannitol, maltose and glucose were intermediate. The third group, having only tartaric acid did not produce any growth.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Grewal (1957) recorded arabinose, lactose and galactose as the best source of carbohydrates for the maximum growth of the fungus. Thind and Sandhu (1956) also confirmed this result with *G. psidii*. Lactose at 2.5-3.0 per cent carbon level gave the maximum growth and sporulation of the fungus.

Of the nine nitrogenous compounds tried best growth and sporulation was observed on peptone and urea, while, nitrates of calcium, potassium and sodium and asparagine supported fair growth. This result is similar to that of Tandon and Grewal (1956) except in case of asparagine which produced better growth than nitrates of potassium and sodium Working with *G. psidii* Thind and Sandhu (1956) got similar results. The growth was relatively poor and colony deformed in all the ammonium sources of nitrogen. The damaging effect of ammonium sources of nitrogen was similar to those observed by Neal, Wester and Gunn (1932) and Blank and Talley (1941) in *Phymatotrichum omnivorum* and Thind and Randhawa (1957) in *Colletotrichum capsici*. Increased levels of nitrogen (urea) resulted directly in increased mycelial output and sporulation with the maximum at 0.15 per cent nitrogen level above which, the growth decreased gradually. Peptone, besides being best source of growth also supported maximum sporulation and largest conidia. Growth was poor in absence of nitrogen which shows the indispensability of this element in nutrition.

Sodium thiosulphate and sodium sulphite yielded maximum growth while sulphates of sodium and potassium seemed to exert inhibitory effect as they produced lesser growth than medium devoid of any sulphur, Sodium bisulphite proved to be toxic to the fungus, as it totally suppressed the growth. Similar toxic effect of this salt has been recorded by Agarwal (1958) in *Fusarium cearuleum*. Magnesium sulphate proved to be the best source of sulphur in producing maximum sporulation. Ammonium sulphate as a sources of nitrogen and sodium bisulphite completely inhibited the sporulation of the fungus. Magnesium sulphate at 0.5-0.6 per cent sulphur level proved best source both for growth and sporulation.

Among various sources of phosphorus tried, dibasic potassium phosphate came out best in all respects viz., growth, extent of sporulation and size of spores. Agarwal (1958) also recorded the maximum growth of *Curvularia penniseti* on dibasic potassium phosphate. The optimum concentration of phosphorus for growth and sporulation varied considerably, the former being maximum at 0.25 per cent and later at 0.15 per cent phosphorus level respectively. Mann (1944) also recorded the rapid and more growth of *Aspergillus niger* in higher phosphorus levels.

Barring few exceptions, the present investigations revealed that sources of carbon, nitrogen, sulphur and phosphorus and their concentrations which supported maximum growth also supported maximum sporulation and length of conidia.

SUMMARY

The carbon, nitrogen, sulphur and phosphorus requirements of *G. musarum* were studied. Lactose, arabinose and galactose proved to be the best sources of carbon for maximum growth of the fungus. Lactose also supported the maximum sporulation. Lactose at 2.5-3.0 per cent

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

carbon levels gave the maximum growth, and sporulation of the fungus.

Amongst the nitrogenous compounds peptone, urea and calcium nitrate supported maximum growth. Sporulation and length of conidia were maximum in peptone. Nitrogen at 0.15 per cent urea level proved best for maximum growth, sporulation and length of conidia.

Sodium thiosulphate and sodium sulphite proved to be the best source of sulphur for maximum growth. Sulphur at 0.05 per cent level supported maximum growth while maximum sporulation occurred at 0.06 per cent sulphur level,

Dibasic and tribasic potassium phosphates proved best for growth while former also supported maximum sporulation, and largest conidia. Maximum growth of the fungus was attained at 0.2-0.4 per cent phosphorus level, but for sporulation it was 0.15 per cent phosphorus level.

ACKNOWLEDGEMENT : The authors are indebted to Sri R. S. Roy, Principal, Bihar Agricultural College, Sabour for invaluable encouragement.

Department of Mycology and Plant Pathology,
Bihar Agricultural College,
Sabour (Bhagalpur),
Bihar.

REFERENCES

AGARWAL, G. P. (1958) An addition to the literature of sulphur and phosphorus requirements of fungi. *Jour. Ind. Bot. Soc.*, **37** : 375-79.

BLANK, L. M. AND P. J. TALLEY (1941) Are ammonia salts toxic to the cotton root-rot fungi ? *Phytopathology* **31** : 926-935.

GREWAL, J. S. (1957) Effect of carbon nutrition on growth and sporulation of some anthracnose fungi. *Ind. Phytopath.*, **10** : 121-25.

MANN, T. (1944) Studies on metabolism of mould fungi I : Phosphorus metabolism in moulds. *Biochem. J.*, **38** : 339-45.

NEAL, D. C., R. E. WESTER AND K. C. GUNN, (1932) Treatment of cotton root-rot with ammonia. *Science*, N. S. **75** : 139-140.

*PEIRIS, J. W. L. (1947) *The Botrytis-disease of gladiolus together with Physiological studies of certain* Botrytis sp. Univ. Lond. Thesis for Ph. D.

THIND, K. S. AND R. S. SANDHU (1956) Studies on nutrition of *G. Psidii*, the incitant of guava anthracnose. *Ind. Phytopath.* **9** : 207-8.

———— AND H. S. RANDHAWA (1957) The influence of different sources of nitrogen on the growth and sporulation of *Colletotrichum capsici*. *Nat. Acad. Sci.*, **27** (B) : 47-52.

TANDON, R. N. AND J. S. GREWAL (1956) Effect of nitrogen nutrition on growth and sporulation of some pathogenic fungi. *Proc. Ind. Acad. Sci.* B., **44** : 61-67.

*Originals not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SEED MICROFLORA II. SEED-BORNE FUNGI OF SETARIA ITALICA, THEIR DISTRIBUTION AND CONTROL

J. S. GREWAL AND MAHENDRA PAL

(Accepted for publication April 10, 1965)

Kangni or fox tail millet (*Setaria italica* Beauv.) is an important crop in the hilly areas of Jammu and Kashmir, Himachal Pradesh, Punjab, Uttar Pradesh, Assam as well as in some parts of Madhya Pradesh, Bihar, Andhra Pradesh, Mysore and Madras. It is grown over an area of 2.5 million acres but the production is only 0.4 million tons. Rust, blast and downy mildew are important diseases of *Setaria italica* but other seed-borne fungi are known to cause considerable damage to the seeds and seedlings in the field. Keeping in view the damage caused by fungi associated with the seed, a collection of *Setaria italica* varieties from different states of India was obtained from Botany Division, Indian Agricultural Research Institute, New Delhi, to assess the distribution and incidence of such seed-borne fungi. In addition, some fungicides were also tested for their comparative efficacy against seed-borne fungal pathogens of *Setaria italica* with a view to recommend the suitable ones to the cultivators.

EXPERIMENTAL : Standard International Rules for Seed Testing[1] were followed throughout these studies. Seeds were tested by agar-plating and blotter methods. In agar-plate method some of the saprophytic fungi like species of *Aspergillus* and *Penicillium* grew very quickly all over the agar plates and did not allow the normal growth of the other fungi but blotter method gave better results, which was, therefore, used in all the experiments in the present investigations.

400 surface sterilized and 400 non-surface sterilized seeds were plated out on moist sterilized blotters in 9.5 cm. Petri-plates in four replicates of 100 at the rate of 25 seeds per plate. Petri-plates were then incubated at 25°C$\pm$1 and were examined for seed-borne fungi 10 days after plating. Surface sterilization was carried out as described by Grewal and Pal (1965)[2].

The data recorded regarding distribution and incidence of various seed-borne fungi on *Setaria italica* in different varieties are summarized in Table I.

It is evident from the Table I that the incidence of seed-borne fungi was less in surface sterilized seeds and it differed with variety or the climatic conditions under which the seeds were produced. In general *Helminthosporium setariae* Saw., *Curvularia lunata* (Wakker) Boedijn, *Rhizoctonia bataticola* (Taub.) Butler as well as species of *Phoma*,

[1]*Proc. Int. Seed Test. Abs.*, **24** : 565-577 (1959)

[2]Grewal, J. S. and Mahendra Pal *Ind. Phytopath.*, **18** : 33-37 (1965)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Incidence and distribution of seed-borne fungi of *Setaria italica* Beauv.

Variety tested	Place of collection		Percentage of the seed infected with						Percentage of Final stand
			Helminthosporium setariae	*Rhizoctonia bataticola*	*Curvularia lunata*	*Phoma* sp.	*Aspergillus* spp.	*Penicillium* spp.	
Koan (I. Se. 26)	West Bengal	S. S.	7.0	4.5	—	—	2.0	—	74.5
		U. S.	13.2	7.5	0.5	—	6.0	—	53.0
Kang (I. Se. 97)	Gujrat	S. S.	—	11.5	6.0	—	—	—	67.5
		U. S.	—	15.2	7.5	3.0	—	—	53.8
Bhedi (I. Se. 96)	Gujrat	S. S.	2.0	7.5	2.0	1.5	—	—	79.5
		U. S.	2.0	10.0	2.2	1.5	—	—	77.5
Karralu (I. Se. 18)	Andhra Pradesh	S. S.	6.5	5.0	—	—	3.0	—	62.0
		U. S.	14.5	5.5	5.0	—	14.0	—	33.5
Koani (I. Se. 56)	Bihar	S. S.	8.5	9.5	3.0	2.0	3.0	—	52.0
		U. S.	9.5	9.5	14.0	4.0	27.0	—	36.0
Tangun (I. Se. 42)	Bihar	S. S.	3.5	2.5	—	—	3.0	1.0	76.5
		U. S.	5.5	2.5	2.5	—	12.0	2.5	61.0
Koan (I. Se. 30)	West Bengal	S. S.	—	—	—	—	2.5	2.5	57.0
		U. S.	—	—	—	—	12.5	4.0	44.0
Shol (I. Se. 112)	Jammu and Kashmir	S. S.	—	—	—	—	50.0	—	40.0
		U. S.	—	—	—	—	62.5	1.5	34.0

S. S. Surface sterilized seeds.
U. S. Unsurface sterilized seeds.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2 : Efficacy of different fungicides on the artificially inoculated and naturally infected seeds of *Selaria italica* Beauv.

Fungicides	Dosage in percentage	Seeds artificially inoculated with						Seeds naturally infected with				
		Helminthosporium setariae		*Curvularia lunata*		*Rhizoctonia bataticola*		*Helminthosporium setariae*	*Curvularia lunata*	*Rhizoctonia bataticola*	Other* fungi	percentage Final Stand
		per cent seed rot	per cent Seedling Blight	per cent seed rot	per cent Seedling Blight	per cent seed rot	per cent Seedling Blight					
Agrosan GN	0.25	—	—	—	—	—	—	—	—	—	—	87.5
Harvesan	0.25	0.5	—	—	—	—	—	—	1.0	—	3.5	76.5
Arasan	0.30	0.75	—	—	—	—	—	—	2.0	—	3.5	74.5
Tritisan	0.25	15.0	44.0	23.5	37.0	19.0	27.5	3.5	12.5	2.0	—	67.0
Panogen	2 ml./kg.	—	—	1.5	2.0	—	—	—	3.0	—	1.0	60.5
Ceresan	0.25	—	—	—	—	—	—	—	0.5	—	1.5	51.0
New Improved Ceresan	0.07	—	—	—	—	—	—	—	—	—	—	46.0
Copper Carbonate	0.30	18.0	19.7	17.0	24.0	2.5	—	6.5	16.5	3.5	28.5	41.5
Rhizoctol	0.25	—	—	30.0	22.0	7.5	—	—	2.0	—	4.5	40.0
Tillex	0.25	—	—	—	—	—	—	—	—	—	—	39.0
Control (inoculated)		40.0	60.0	36.5	49.0	23.0	48.0	—	—	—	—	—
Control (uninoculated)		—	—	—	—	—	—	8.0	16.25	6.0	33.5	34.0

*Other fungi include spp. of *Aspergillus*, *Penicillium* and *Phoma*.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Aspergillus and *Penicillium* were found to be associated with the seeds. It was further observed that the first three fungi caused seed rot and seedling blight, fourth one caused seedling blight alone while the last two reduced germination of the seeds to some extent. *Setaria italica* var. Bhedi (I. Se. 96), Tangun (I. Se. 42) and Koan (I. Se. 26) respectively supported higher final stand of healthy seedlings as compared with rest of the varieties. Variety Shol (I. Se. 112) from Jammu and Kashmir was free from all pathogens except *Aspergillus* sp.

In comparative efficacy tests with various fungicides, seeds of *Setaria italica* var. Kang (I. Se. 97) were artificially inoculated with *H. setariae*, *C. lunata*, and *R. bataticola* separately in three lots and in addition, naturally infected seeds of var. Koani (I. Se. 56) were treated with following fungicides. Agrosan GN (tolylmercury acetate), Harvesan (organically combined mercury), Arasan [bis (dimethylthiocarbamoyl) disulphide], Tritisan (pentachloronitrobenzene), Panogen [cyano (methylmercuri) guanidine], Ceresan (phenylmercury acetate), New improved ceresan (ethylmercury phosphate), Copper Carbonate (copper carbonate), Rhizoctol (methylarsinic sulphide), and Tillex (ethylmercury chloride). The treated seeds were plated on sterilized moist blotters with a view to see the effect of various fungicides on the above three pathogens individually as well as in combination in naturally infected seeds. The data recorded are summarized in Table II.

It is evident from the Table II that Panogen was less affective against *Curvularia lunata* and Rhizoctol against *C. lunata* and *Rhizoctonia bataticola* whereas Tritisan and Copper carbonate were not affective against any seed-borne fungus. Seed treatment with Agrosan GN, New Improved Ceresan and Tillex checked the growth of all seed-borne fungi completely. Final stand in New Improved Ceresan and Tillex, however, was less as compared with that on Agrosan GN, perhaps due to phytotoxicity. Harvesan and Arasan controlled seedling blight completely and reduced the seed rot due to *H. setariae* to 0.5 and 0.75 per cent respectively as against 40 per cent in artificially inoculated control.

Agrosan GN controlled the seed-borne fungi completely and supported highest final stand of 87.5 per cent as against 34.0 per cent in untreated naturally infected control. Therefore, seed treatment with Agrosan GN at the rate of 2.5 gms/Kg. can be recommended for the control of seed-borne fungi of *Setaria italica*.

SUMMARY

Eight different varieties of *Setaria italica* Beauv. collected from five States of India were tested for seed-borne fungi by standard International Seed Testing Methods. It was observed that *Helminthosporium setariae*, *Curvularia lunata* and *Rhizoctonia bataticola* caused seed rot and seedling blight, while Species of *Aspergillus* and *Penicillium* only reduced seed germination. Variety Bhedi (I. Se. 96) from Gujrat, Tangun (I. Se. 42) from Bihar and Koan (I. Se. 26) from West Bengal respectively supported higher final stand as compared with rest of the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

varieties. Out of the ten fungicides (namely: Agrosan GN, Harvesan, Arasan, Tritisan, Panogen, Ceresan, New Improved Ceresan, Copper Carbonate, Rhizoctol and Tillex) tested for their comparative efficacy against seed-borne fungi of *Setaria italica* seed treatment with Agrosan GN at the rate of 2.5 gm/Kg. controlled all seed-borne fungi completely and supported the highest final stand of healthy seedlings and, therefore, can be recommended as standard seed treatment for the control of seed-borne fungi of *Setaria italica*.

ACKNOWLEDGEMENTS : The authors are thankful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for his keen interest and providing necessary facilities.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TESTS WITH DIFFERENT VARIETIES OF SESAMUM FOR RESISTANCE TO PHYTOPHTHORA BLIGHT OF SESAMUM

P. D. GEMAWAT[1] AND N. PRASAD[2]

(Accepted for publication April 10, 1965)

Earlier studies by Kale (1954) and Kale and Prasad (1957) have shown that blight of sesamum (*Sesamum orientale* L.) causes severe losses to the crop. Not much work has been done in connection with the evolving varieties of sesamum resistant to diseases Crandall and Dieguez (1948) found all the selections of *S. orientale* tested to be susceptible to *Phytophthora cactorum* causing stemcanker.

Seed of different varieties of sesamum were obtained from different parts of India and they were tested for resistance to *Phytophthora parasitica* var. *sesami* Prasad. Since Kale and Prasad (1957) reported that the plants were liable to get infection at any stage after the seedlings were about one week old, testing at the seedling stage (3 weeks) and adult stage (6 weeks) in the greenhouse as well as in the field were carried out.

For greenhouse testing, seeds of different varieties were sown in clay pots sterilized with 5 per cent formalin sloution and filled with sterilized soil. One week old seedlings were thinned out to keep six plants in each pot. In all cases the innoculations were carried out at the collar region with a 10-day-old culture grown on oat meal agar. The surviving plants were again inoculated a week after first inoculation. In the field trial, the varieties were tested under natural conditions of infection which were very favourable for disease production. Observations were recorded on the extent of lesions on the stem, on number of leaves infected (adult stage) and the percentage of plants diseased.

RESULTS: The length of the stem lesions per plant 15 days after inoculation, in case of seedling and the adult plant tests carried out in the greenhouse ; the number of leaves showing infection in the adult stage and the percentage of plants infected in field trial are represented in Table 1. From the table it is clear thrt 38 varieties out of the 41 tested in the greenhouse in the seedling and the adult stages were highly susceptible to the pathogen and that all the plants were badly infected within two weeks of inoculation. Only three remaining pure-line strains, which survived were given two more weekly inoculations and it was found that, except strain No. 75 A/1—1/2—1, the other two viz., 58/1—1/2—2/1 and 23A/1—1/1—2/1 also exhibited symptoms of the disease within one week. Even the resistant strain No. 75 A/1—1/2—1 showed signs of infection at

[1]Vijnan Mandir Officer, Dabok (Udaipur), Rajasthan.

[2]Dean, S. K. N. College of Agriculture, Jobner, University of Udaipur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

maturity. In the adult-stage too the results were same. It may therefore be inferred that the pathogen is very virulent and that the strains Nos. No. 58/1—1/2—2/1 and 23 A/1—1/1—2/1 may be considered as tolerant to the disease and the strain No. 75 A/1—1/2—1 may be treated as resistant. In the field trials all the 33 varieties tested were found to be susceptible.

TABLE 1 : Performance of different varieties and strains of sesamum against *P. parasitica* var. *sesami*.

Name of the variety of strain used	Source	Stem lesions per plant in cms.		No. of leaves infected per plant in adult trial	percentage infection in field trial	Remarks
		seedling	adult			
1	2	3	4	5	6	7
Pure inbred lines : S-5						
3A/4—2/1—1/1	Economic Botanist,	5.5	5.0	2	—	—
10/2—1/2—1/1	Kanpur	7.0	6.75	3	—	—
13/1—2/2—2/1	do	slight	5.0	Nil	—	—
45/2—1/1—1/1	do	16.3	7.5	3	—	—
50A/1—1/1—2/1	do	5.3	5.0	5	—	—
I.C.C.-B/1—1/1—1/1	do	7.0	5.25	2	—	—
58/1—1/2—2/1	do	0.7	0.66	1	—	Tolerant
I.C.C.-C/2—2/1	do	4.0	2.0	2	—	—
20/2—1/1—1/1	do	3.0	2.0	2	—	—
23A/1—1/1—2/1	do	slight	2.5	1	—	Tolerant
90A/1—1/1—1/1	do	6.0	6.25	5	—	—
94A/1—1/2—1	do	5.0	7.75	6	—	—
75A/1—1/2—1	do	No	slight	Nil	—	Resistant
85A/2—2/2—2	do	3.0	10.0	2	—	—
48B—1/2—2/1—2/1	do	4.0	4.0	2	—	—
Open pollinated						
T-10	do	3.5	3.8	2	25.4	
48A/5	do	4.0	3.0	2	—	
T. 65	do	4.4	2.0	1	8.4	

Contd.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Contd.

1	2	3	4	5	6	7
I. C. C. A.	Eco. Bot. Kanpur	3.5	2.5	1	12.5	
63 B	,,	6.0	5.5	2	13.8	
80 B-2-1	,,	2.5	2.4	5	9.1	
Kanpur Local	,,	2.0	4.0	5	14.0	
48 A/5-1	,,	4.4	7.0	4	15.4	
S.I. 864, Edapadi	Eco. Bot. Madras	4.5	12.0	8	22.8	
S. I. 929, Salur Punasa	,,	4.6	8.5	9	24.0	
S. 934, Yellaman-chilli Punasa	,,	7.1	9.5	5	32.0	
S. I. 948, Multhalai	,,	4.7	10.0	9	27.2	
S. I. 953, Sirayam	,,	5.2	6.75	3	20.0	
S. I. 951, Irumboor-thipathi	,,	5.2	7.8	2	11.6	
N. P. 6	I.A.R.I. New Delhi	3.0	5.6	2	37.2	
N. 41 Kharif	Eco. Bot. Nagpur	4.8	2.1	2	12.8	
N. 128, Kharif	,,	4.5	2.1	1	10.0	
N. 8, Rabi	,,	6.0	3.0	2	8.2	
N. 37, Rabi	,,	6.5	2.0	2	20.0	
N. 85, B. S. (Improved)	D.A.O. Nasik	4.8	1.8	1	10.9	
Jambusari Tal	D.A.O. Broach	8.0	5.6	3	13.3	
Local Tal (Black)	Agri. Ins. Anand	5.2	6.0	4	18.8	
Local Tali (White)	,,	5.6	8.0	4	42.8	
Wild species	N. C. Farm Chharodi	3.5	10.0	4	No growth	
Kutch local, Kharif	Kundrodi	3.8	5.0	4	—	
Kutch Lal, Rabi	,,	4.0	3.0	2	—	

Contd.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

1	2	3	4	5	6	7
N. P. 7	I.A.R.I. New Delhi	—	—	—	25.0	
N. P. 3	,,	—	—	—	12.1	
Local Til	Parmar, Sanjaya	—	—	—	25.0	
Local Til	Patel, Sanjaya	—	—	—	12.0	
Local Til	Babubhai Sanjaya	—	—	—	10.6	
Local Til	N. M. Patel Sanjaya	—	—	—	11.8	
Local Til	P. D. Patel Samarkha	—	—	—	17.7	
Gingelly T.M. V. 1	Coimbatore	—	—	—	21.0	
Gingelly T.M.V. 2	Coimbatore	—	—	—	23.3	
Gingelly T.M.V. 3	Coimbatore	—	—	—	25.0	

DISCUSSION : The present studies conducted in the greenhouse and field with different varieties of sesamum against *P. Parasitica* var. *sesami* show that as in case of late blight of potato (*P. infestans*) or black shank of tobacco (*P. parasitica* var. *nicotianeae*) no immune variety is available which combines with it all the other desirable characters (Foster-1943, Kincaid-1949, Valleau-1952). Out of 41 varieties of sesamum tested in green-house against the disease (*P. parasitica* var. *sesami*), not even one was found to be immune. One black seeded pure line strain 75 A/1—1/2—1 showed some resistance, whereas two other white-seeded pure line strains 58/1—1/2—2/1 and 23 A/1—1/1—2/1 were observed to possess tolerance to the disease. But in later years the plants raised from selfed seeds of these particular plants were found to be susceptible. All other varieties were susceptible. Even the wild species, *S. lacinatum* was heavily infected. All the 33 strains and varieties tested in the field were also found to be susceptible.

SUMMARY

Fifty one sesamum varieties were tested for their resistance to *Phytophthora* blight (*P. parasitica* var. *sesami*). In the greenhouse studies 41 varieties were tested in the seedling and adult stages. A field trial with 33 different varieties and strains of sesamum was also carried out.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Out of 41 varieties and strains tested in the greenhouse, one strain (75 A/1—1/2—1) was found to be resistant and two others viz. 58/1—1/2—2/1 and 23 A/1—1/1—2/1 showed some tolerance against the disease, while all other were susceptible. But in subsequent years plants raised from selfed seed from these particular plants also were found to be susceptible.

In the field all the 33 different varieties tested were susceptible.

ACKNOWLEDGEMENTS : The authors are indebted to Economic Botanist (Oilseeds), Uttar Pradesh ; Economic Botanist, Madhya Pradesh ; Deputy Director (Crop Research) erstwhile Bombay State and Professor of Botany, Institute of Agriculture, Anand, for the supply of seeds. Sincere thanks are also due to Dr. M. D. Patel, Director, Institute of Agriculture, Anand (Gujarat) for the facilities to carry out the work.

Institute of Agriculture,
Anand (Gujarat)

REFERENCES

CRANDALL, B. S. AND J. DIEGUEZ (1948) *Phytophthora* stem-canker of sesamum in Peru. *Phytopathology* **38** : 753-755.

FOSTER, H. H. (1943) Resistance in the Genus *Nicotiana* to *Phytophthora parasitica* Dastur var. *nicotianeae* Tucker. *Phytopathology* **33** : 403-404.

KALE, G. B. (1954) *Studies on the blight of sesamum.* M. Sc. (Ag) Thesis, Gujarat University (Unpublished).

KALE, G. B. AND N. PRASAD (1957) *Phytophthora* blight of sesamum *Indian Phytopath.* **10** : 38-47.

KINCAID, R. R. (1949) Three interspecific hybrids of tobacco. *Phytopathology* **39** : 284-287.

VALLEAU, W. D. (1952) Breeding tobacco for immunity to Black shank. Abs. in *Phytopathology* **42** : 288.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MUTATION IN COLLETOTRICHUM FALCATUM THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE V. PATHOGENICITY OF BIOCHEMICAL MUTANTS

B. S. BAJAJ,* M. S. CHATRATH AND R. S. VASUDEVA

(Accepted for publication April 10, 1965)

The red rot fungus (*Colletotrichum falcatum went*) of sugarcane has been subjected to induced mutation with different kinds of ionizing radiations and a variety of mutants, particularly those showing change in biochemical characters, have been isolated (Vasudeva *et al.* 1957; Bajaj and Chatrath 1958, 1960; Bajaj, Chatrath and Vasudeva, 1964). Further studies on the pathogenicity of the induced biochemical mutants on sugarcane have been made with a view to understand the nature of virulence in the red rot pathogen and the results obtained are reported here.

MATERIAL AND METHODS : The biochemical mutants used in these studies were obtained by irradiation of the wild type strain (No. 244) of *C. falcatum* with radio-active phosphorus in growth phase. The method of irradiation, dosages and procedure for mutant-screening have already been reported (Bajaj, Chatrath and Vasudeva 1964). Twelve mutants showing specific deficiency for one or more vitamins were selected from three different experiments viz. X (241 μc/ml.), U (272 μc/ml.) and M(400 μc/ml.) and mutants were designated according to the experiment. The wild type strain (No. 244) was also included in the pathogenicity tests as control. The following mutants were used in these studies :—

Mutant	*Morphological Type*	*Deficiency*
X_{11}	Dark	p-aminobenzoic acid
U_7	Light	Biotin
M_1	Dark	Biotin
M_2	Dark	Biotin
M_3	Dark	Mixed vitamins
M_4	Dark	Biotin and choline
M_5	Dark	Thiamine
M_6	Light	Thiamine and choline
M_8	Light	Thiamine
M_9	Light	Thiamine
M_{10}	Dark	Thiamine
M_{11}	Light	Thiamine
Cf 244 (Wild type)	Light	Prototrophic

*Present address :—Deputy Superintendent, Antibiotic Project, Virbhadra, Rishikesh (U. P.).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

All the cultures were purified by single spore isolations and maintained on oat meal agar. For multiplication of inoculum, the cultures were plated on oat meal agar and spores or mycelium harvested, washed twice aseptically by centrifugation and resuspended in sterile distilled water. Heavy concentration of spore or mycelial inoculum was used in all cases. Pathogenicity of all the mutants was tested by inoculating standing canes in pots, by the plug method (Chona, 1954), using spore or mycelial suspension as inoculum. For this purpose, selected clumps of sugarcane variety Co. 647 were transplanted from fields of Agronomy Division of the Institute into 30 cm. diameter earthen pots during the month of September so as to get canes of uniform size for inoculation.

Final observations were recorded by splitting open the inoculated canes longitudinally and measuring the linear spread of infection. Reisolations of different mutants from the inoculated canes were made from the junction of healthy and diseased portions of the cane tissue on oat meal agar slants. As soon as the cultures were about 10 days old, these were purified by establishing single spore culture. The identity of these cultures was confirmed by testing their deficiency on final selective medium (Bajaj, Chatrath and Vasudeva 1964). Their morphological characters were also compared with the original cultures.

EXPERIMENTAL : In order to test the pathogenicity of twelve selected biochemical mutants of *C. falcatum*, 25 standing canes of variety Co. 647, were inoculated as above. Inoculations were also made with the wild type strain (No. 244) for comparison of virulence. Periodic observations on the progress of red-rot disease were made by splitting open one or two canes from the set inoculated with the wild type. Final observations were recorded after 2 months of inoculations. The mutants were classified into 3 categories: pathogenic (P), partially pathogenic (PP), and non-pathogenic (NP), on the basis of linear and lateral spread of infection and type of red-rot symptoms produced. A mutant was considered pathogenic if it produced typical red rot symptoms, covering at least one internode on both sides of inoculated internode. The spread of infection in such cases was usually more than 40 cm. The mutants producing infection in the form of thick streaks, covering atleast one internode on either or both sides of the inoculated internode, with linear spread usually between 20-40 cm. were classified as partially pathogenic. In the category of non-pathogenic mutants, all those cultures which produced linear infection usually less than 20 cm. in the form of a few isolated streaks and generally confined only to the inoculated internode, were included. The results of linear spread of infection alongwith pathogenicity rating are given in Fig. 1.

It would be observed from the figure that the biochemical mutants which were morphologically classified as dark type were either non-pathogenic or partially pathogenic, irrespective of the type of deficiency.

On the other hand all the biochemical mutants resembling light type cultures in morphological characters were either rated as pathogenic or partially pathogenic, though these showed deficiency for different growth factors.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

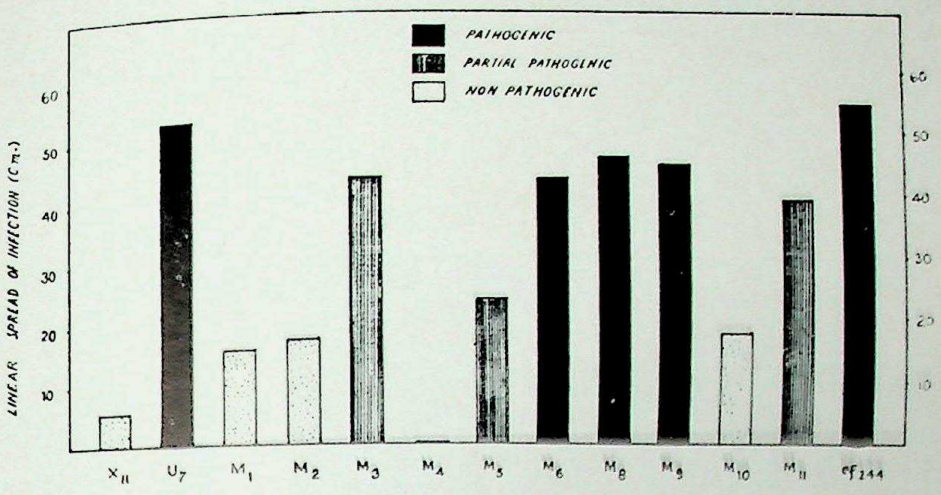


Fig. 1. Comparative virulence of different biochemical mutants of *Colletotrichum falcatum* on sugarcane variety Co. 647.

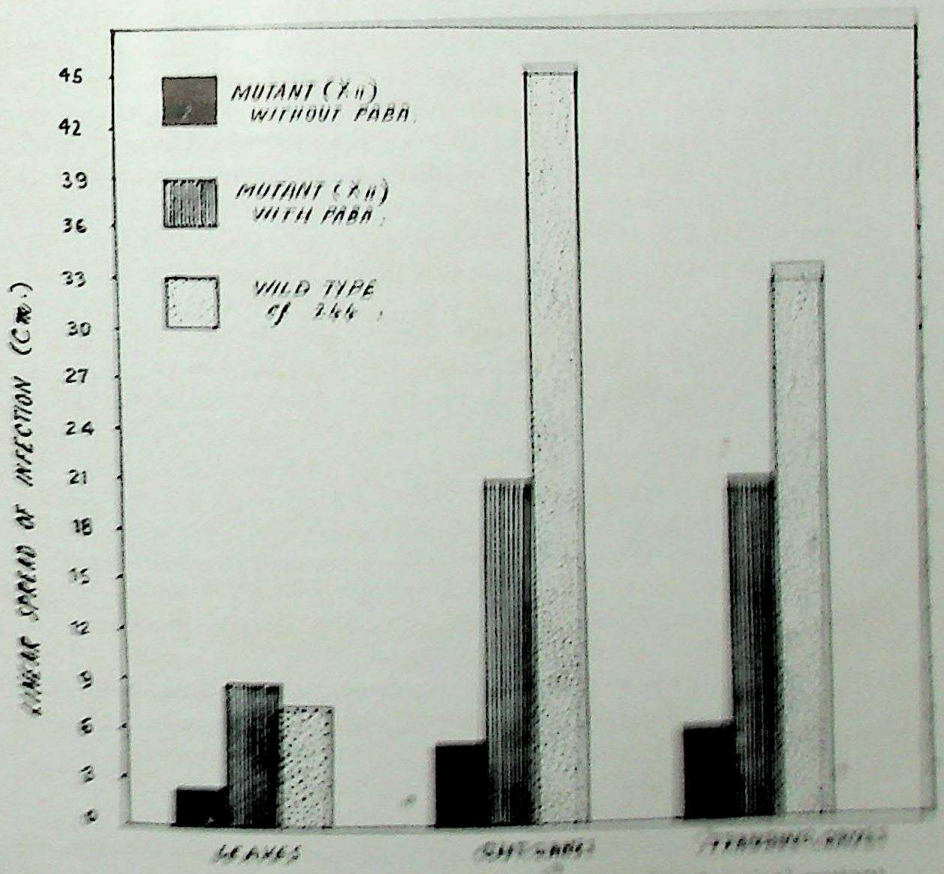


Fig. 2. Restoration of pathogenicity of the biochemical mutant (X_{11}) by feeding the canes with PABA

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

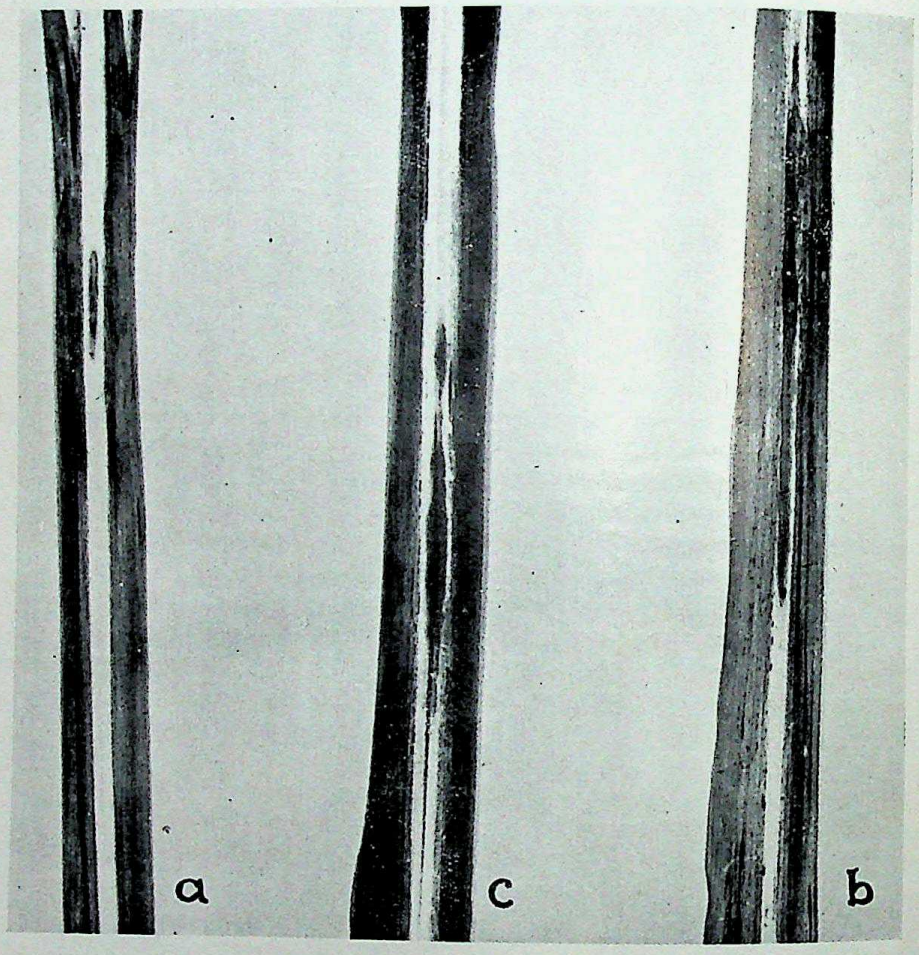


Fig. 3. Restoration of pathogenicity of the biochemical mutant (X_{11}) by feeding the plants with PABA through roots.

(a) Mutant strain showing restricted spread on leaf midrib of sugarcane.

(b) Mutant strain showing increase in linear spread of red rot on leaf midrib of the plant fed with PABA through roots.

(c) Wild type (without feeding).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Bacon, Burrows and Yates (1950) showed that induced biochemical mutation with known nutritional deficiencies was associated with change in pathogenicity. In the case of *Bacterium typhosum*, a mutant requiring p-aminobenzoic acid (PABA) was found to be avirulent. In the present study a mutant of *C. falcatum* requiring PABA, which has been found to be non-pathogenic on sugarcane variety Co. 647, is considered interesting for the study of pathogenesis of red rot disease. In order to further study the effect of PABA deficiency in the mutant on its pathogenicity on sugarcane and the effect of incorporation of PABA in the host, experiments were set up where cut and standing canes as also the leaves of variety Co. 647, were inoculated. The pathogenicity test on leaves of sugarcane was conducted by inoculating leaf mid-rib of young-potted plants by the method described elsewhere (Chona, Bajaj and Sharma, 1964). The inoculated plants were incubated for 15 days to allow the disease to progress and the final observations were recorded by measuring the linear spread of infection on the mid-rib.

The pathogenicity experiment was also conducted with cut canes of uniform size using the plug method of inoculation. The final observations were recorded after about a month in the same way as described for standing canes.

As far as the feeding of plants with growth factors was concerned, the pots were periodically irrigated with vitamin solutions, keeping controls with distilled water only. In the case of standing canes and leaves, the PABA solution was applied to the soil at the rate of 1 mg. and 250 μg., respectively per pot on every alternate day. In the case of cut canes, the vitamin was incorporated into the canes along with the inoculum at the rate of 0.75 μg. per cane.

The results of various experiments conducted in this connection are illustrated in Fig. 2. The infection and symptoms on leaves of sugarcane are shown in Fig. 3.

These observations clearly indicate that the loss of the ability of the mutant to synthesize PABA has resulted in its loss of pathogenicity on sugarcane variety Co. 647 and that its parasitic vigour can be restored partly by making available the required growth factor in the host system. Reisolations made from the internodes other than inoculated ones, as also from the infected leaves, particularly in those treatments where PABA was incorporated in the host and inoculated with mutant strain, yielded typical cultures of the biochemical mutant deficient in PABA.

Studies on the growth factor requirement of the mutant and its pathogenicity indicate that loss of virulence of mutant is governed by the non-availability of the growth factor (PABA) in the host variety Co. 647. This was further confirmed by extracting the juice of cane variety Co. 647 in acid cleaned pyrex Petridishes and incorporating the same in agar so as to get 10 per cent concentration of sugarcane juice. As the mutant does not show any growth on DAP medium, it was not added in the cane juice agar. The controls were maintained

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

on Dextrose-Asparagine-Phosphate medium (DAP*) containing optimum concentration of PABA. The spores of the mutant, after twice washing by centrifugation, were seeded by spot inoculation method and observations on growth recorded after 10 and 15-day incubation. Mycelial growth and sporulation was restricted to the point of inoculation in the case of cane juice agar medium showing thereby that PABA is either not available in sugarcane juice of variety Co. 647 or available only in concentration much lower than those required for the growth of the mutant. The mutant, however, showed normal growth on DAP containing PABA medium.

DISCUSSION : Pathogenicity tests of different biochemical mutants of *C. falcatum* revealed that mutants deficient in PABA (X_{11}), biotin (M_1 and M_2), biotin and choline (M_4) and thiamine (M_{10}) were non-pathogenic on sugarcane variety Co. 647; those deficient in thiamine (M_5 and M_{11}) and certain other unidentified growth factors (M_3) were partially pathogenic while mutants deficient in biotin (U_7), thiamine (M_8 and M_9), thiamine and choline (M_6) were as pathogenic as the wild type. These results show that loss of ability of an organism to synthesise particular metabolites does not always result in loss of pathogenicity. It is further observed that mutants having similar deficiency do not always show the same trend in pathogenicity. Though no attempt was made to analyse the cause of biotin or thiamine deficient mutants being either virulent or avirulent on sugarcane variety used in this study, but since the required nutrilites, i.e. biotin and thiamine, are available in the host in low concentration (Brinkley and Wolform 1953), it is possible that two mutants deficient for the same growth factor may require different concentrations for their growth.

As the host serves as growth medium for the metabolism of the parasite, it is essential that the nutrilites required by the pathogen for its growth must be available in the host or in other words the host should provide adequate nutritional environment (Garber 1956). Most of the biochemical mutants of *Venturia inaequalis* (Boone, Kline and Keitt, 1957), *Salmonella typhosa* (Bacon *et al.*, 1950 and 1951), *Klebsiella pneumoniae* (Garber *et al.*, 1952), *Erwinia aroideae* (Garber, 1954), *Agrobacterium tumefaciens* (Garber *et al.*, 1955), which showed loss of pathogenicity on their respective hosts, were non-pathogenic either because the required nutrilite was not present in the host or if present, was not available at the site of infection. In the present study, the biochemical mutant deficient in PABA showed very restricted growth on the cane-juice agar medium showing that loss of pathogenicity on this variety was also due to nonavailability of the growth factor or in other words due to 'inadequate nutrition'. By incorporating PABA in the host system, through the roots or through inoculum, the virulence of the mutant could be partly restored both on leaf midrib and cane stalk. The reisolations made from the infected tissue revealed that the mutant was still deficient in PABA and had not reverted to prototrophy. These results further confirm that loss of pathogenicity in this case is due to non-availability of growth factor, i.e. PABA, in the host variety. This

*Dextrose 30 g., Asparagine 1.0 g., $MgSO_4$ 0.5 g., M/30 phosphate buffer at pH 6.0.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

supports Garber's hypothesis (1956) that adequate or inadequate nutrition available in the host would determine whether the pathogen will be virulent or avirulent, subject to the inhibitory environment being ineffective. It has thus been possible to control experimentally the pathogenicity of an organism by supplying or withholding the required nutrilite from the host.

SUMMARY

Studies conducted on the pathogenicity of induced biochemical mutants of *Colletotrichum falcatum* Went, the red rot organism of sugarcane with a view to understand nature of virulence in the pathogen showed variation from completely non-pathogenic to as pathogenic as the wild type. It was observed that loss of ability of the organism to synthesise a particular metabolite does not always result in the loss of pathogenicity and the mutants having similar deficiency do not always show the same trend of pathogenicity.

The biochemical mutant deficient in p-aminobenzoic acid was found to be completely avirulent on sugarcane variety Co. 647 and since it did not show any growth on sugarcane juice of same variety, it was concluded that the loss of virulence in this case was due to the non-availability of growth factor (inadequate nutrition) in the host. This was further confirmed by incorporating the vitamin (PABA) in the host system which resulted in partial restoration of pathogenicity.

ACKNOWLEDGEMENTS : The authors are thankful to Dr. B. L. Chona, Head of the Division for critically going through the manuscript and valuable suggestions.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

REFERENCES

BACON, G. A., T. W. BURROWS AND MARGARET YATES (1950) The effect of biochemical mutation on the virulence of *Bacterium typhosum* : The virulence of mutants. *Brit. Jour. Exptl. Pathol.* **31** : 714-724.

BACON, G. A., T. W. BURROWS AND MARGARET YATES (1951) The effect of biochemical mutation on the virulence of *Bacterium typhosum*: The loss of virulence of certain mutants. *Brit. Jour. Exptl. Pathol.* **32** : 85-96.

BAJAJ, B. S. AND M. S. CHATRATH (1958) Some useful applications of ionizing radiations in plant pathological research: *Proc. Mycol. Res. Wkrs. Conf.*, Simla. Indian Council of Agricultural Research, New Delhi.

BAJAJ, B. S. AND M. S. CHATRATH (1960) Growth phase method for mutant-screening in *Colletotrichum falcatum* Went. *Indian Phytopath.* **13** : 156-160.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BAJAJ, B. S., M. S. CHATRATH, AND R. S. VASUDEVA (1964) Mutation in *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane. IV. Morphological and biochemical mutants induced by ionizing radiation. *Indian Phytopath.* **17** : 296-303.

BOONE, D. M., D. M. KLINE AND G. W. KEITT (1957) *Venturia inaequalis* (Cke.) Wint. XIII. Pathogenicity of induced biochemical mutants. *Amer. Jour. Botany.* **44** : 791-796.

BRINKLEY, W. W. AND M. L. WOLFORM. (1953) Composition of cane juice and cane final molasses. Sugar Research Foundation, Inc. New York. *Scientific Report Series No.* **15** : 1-24.

CHONA, B. L. (1954) Studies on the diseases of sugarcane in India IV. Relative resistance of sugarcane varieties to red rot. *Indian Jour. Agric. Sci.* **24** : 301-316.

CHONA, B. L., B. S. BAJAJ AND RAJESHWARI SHARMA (1964) Perithecial production by different strains of *Glomerella tucumanensis* (Speg.) Arx and Mueller. *Indian Phytopath.* **17** : 241-244.

GARBER, E. D. (1954) The role of nutrition in the host-parasite relationship. *Proc. National Acad. Sci.* (U. S.), **40** : 1112-1118.

GARBER, E. D. (1956) A nutrition inhibition hypothesis of pathogenicity. *Amer. Nat.* **90** : 183-194.

GARBER, E. D., M. GOLDMAN AND G. SUSAN HIRSHHORN (1955) Auxotrophy and virulence in *Agrobacterium tumefaciens* (Abs.) *Bacteriol. Proc.* 122.

GARBER, E. D., ADELINE J. HACKETT AND R. FRANKLIN (1952) The virulence of biochemical mutants of *Klebsiella pneumoniae. Proc. National Acad. Sci.* (U. S.) **38** : 693-697.

VASUDEVA, R. S., B. S. BAJAJ, M.R.S. IYENGAR, M. S. CHATRATH AND P. L. GANJU (1957) Mutagenic and stimulative effects of ionizing radiations on certain micro-organisms. *Proc. 4th Internat. congress on crop Prot.* Hamburg, Vol. **1** : 121-123.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES OF MOSAIC DISEASE OF ELEUSINE IN THE DECCAN

D. G. RAO, P. M. VARMA AND S. P. CAPOOR

(Accepted for publication April 19, 1965)

INTRODUCTION : In June 1961, three to five per cent plants of *Eleusine aegyptia* (Linn.) Desf. [Syn. *Dactyloctenium aegyptiacum* Willd. or *D. aegyptium* (L.) Beauv.] and *Eleusine indica* (L.) Gaertn, growing along river banks at Poona were found infected with mosaic. Further, during August and September 5 to 8 per cent infection was noted in cultivated Ragi, *Eleusine coracana* Gaertn. Investigations were taken up to establish the identity of the disease, study its host range with particular reference to *Zea mays* L., *Sorghum vulgare* Pers., *Setaria italica* Beauv. and other common grasses of economic importance, and also study the properties of the virus and its transmission. The results of these investigations are discussed in this paper.

Species of *Eleusine* have been recorded as collateral or experimental hosts of sugarcane mosaic virus by Brandes and Klaphaak (1923), who showed that *Eleusine coracana* could be infected through *Rhopalosiphum maidis* Fitch., while Kunkel (1924) suspected *E. indica* to be infected with sugarcane mosaic virus in Hawaii. In India, McRae (1929) and Venkatarayan (1946) recorded mosaic infection in *E. coracana* from Vizagapatam (Andhra State) and Mysore State, respectively. Chona and Seth (1960) reported transmission of maize mosaic virus to *Dactyloctenium aegyptium*. Except that these species were shown to be susceptible to sugarcane mosaic and maize mosaic viruses, no serious attempt seems to have been made to study the natural spread and the range of susceptible host plants of the mosaic disease of *Eleusine* species reported in this paper.

MATERIAL AND METHODS : The virus for these experiments was collected from infected plants of *Eleusine aegyptia*, *E. indica* and *E. coracana*, and its culture was maintained in *E. coracana* and *E. indica* by repeated inoculations.

All experimental plants were raised in insect proof glasshouse from seeds. Cent per cent infection resulted in *Eleusine* spp. only when the test plants at the time of inoculation were 15 to 30 days old. High percentage of infection in *Zea mays*, *Sorghum vulgare*, and *Euchlaena mexicana* Schrad. was obtained when 3-to 5-day old plants were inoculated.

The inoculum was prepared by grinding young leaves of diseased plants in distilled water with Carborundum and straining through muslin. Manual inoculation was done by the method described by Rawlins and Tompkins (1936) using 300-mesh Carborundum as abrasive. Excess inoculum was washed off from the leaves with water. By this method

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

cent per cent infection was produced in the inoculated plants, while only 45-52 per cent plants were infected when inoculated by the methods used by Sein (1930) and Matz (1933).

INOCULATION WITH APHIDS :

The undermentioned aphid species were tested for transmission of the virus :

1. *Rhopalosiphum maidis* Fitch : This species was collected from the aerial parts of *Eleusine coracana*, *E. indica* and *E. aegyptia*.

2. *Tetraneura nigriabdominalis* Sasaki : This aphid was collected from the roots of the three *Eleusine* species.

3. *Aphis gossypii* Glover : This species was collected from cotton, Bhendi and egg plant, the common food plants of the aphid and is referred to as the *Common strain*. It was also collected from *Colocasia esculenta* Schott and is called the *Colocasia strain*, and lastly from *Commelina benghalensis* L. and *C. nudiflora* L. The last one has been distinguished as the *Commelina strain*. The *Colocasia* and the *Commelina strains* are physiologically and morphologically different from the *Common strain* of *A. gossypii* and are also distinct from each other.

4. *Aphis fabae* Scap : This aphid was collected from *Trianthema portulacastrum* L.

5. *Longiunguis sacchari* Zehntner : This species was collected from *Saccharum officinarum* L.

6. *Myzus persicae* (Sulz.) : This was collected from *Brassica pekinensis* Rupr.

7. *Schizaphis cyperi* (V. D. G.) : This aphid was collected from *Cyperus rotundus* L.

8. *Toxoptera* (*Schizaphis*) *graminum* (Rond.) : This species was collected from *Triticum aestivum* L.

9. *Pentalonia nigronervosa* Coq : The banana aphid was collected from *Musa cavendishii* Lamb.

The aerial feeding aphids were starved for 2-3 hours before feeding them for a short period of 8-10 minutes on source plants. These were then liberated on test plants for 18-24 hours. All the feeding of aphids was carried out in dark in order to ensure the maximum transmission. After the feeding was over, the aphids were killed by spraying the plants with 0.01 per cent Folidol.

Since some difficulty was experienced in experiments with the root aphid, *Tetraneura nigriabdominalis*, special technique was devised for inoculating experimental plants by this aphid species.

For culturing the aphids on roots of diseased or healthy plants, only grown-up plants with a large number of fibrous roots were used.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

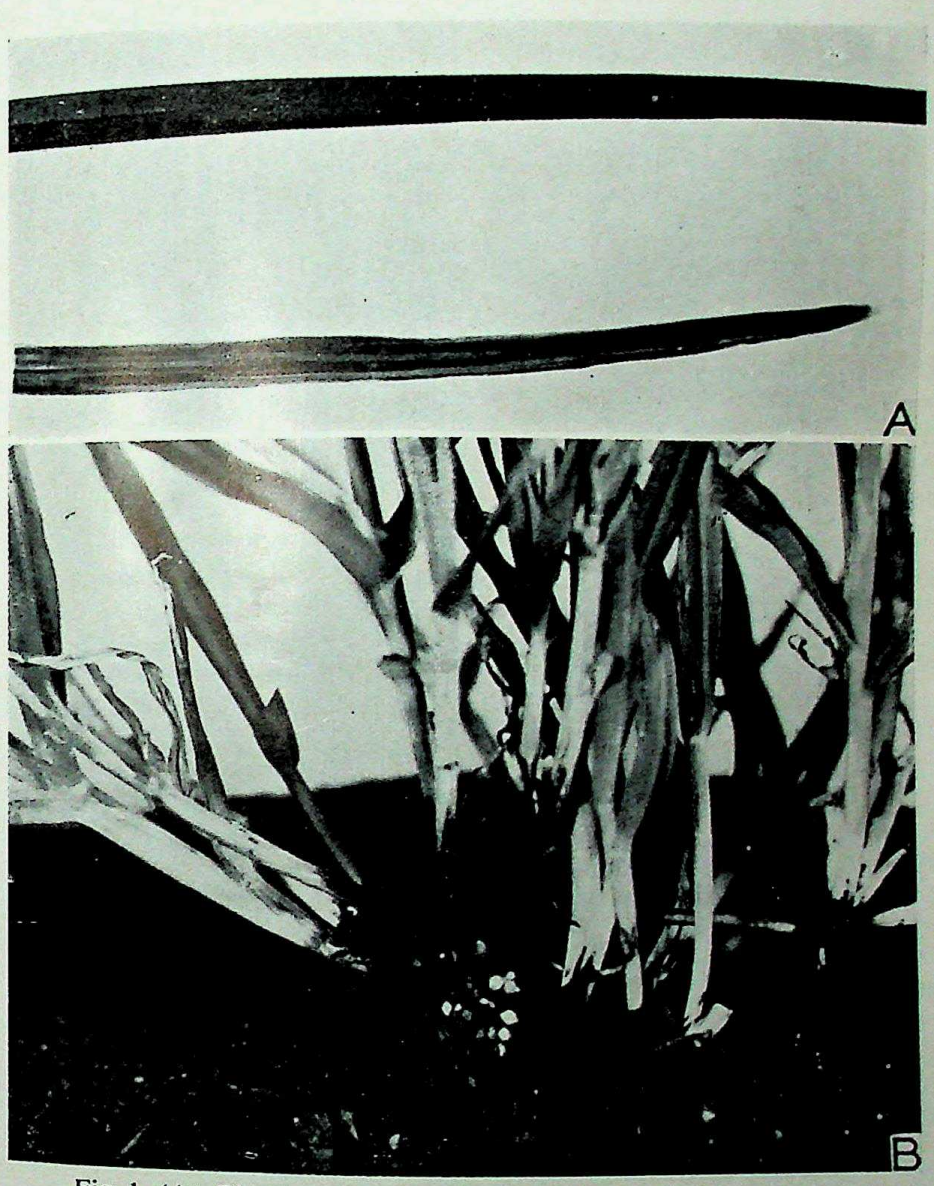


Fig. 1. (A) *Eleusine coracana* leaf showing typical mosaic symptoms. Healthy leaf on top (B) A colony of *Tetraneura nigriabdominalis* on exposed roots of *Eleusine coracana*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

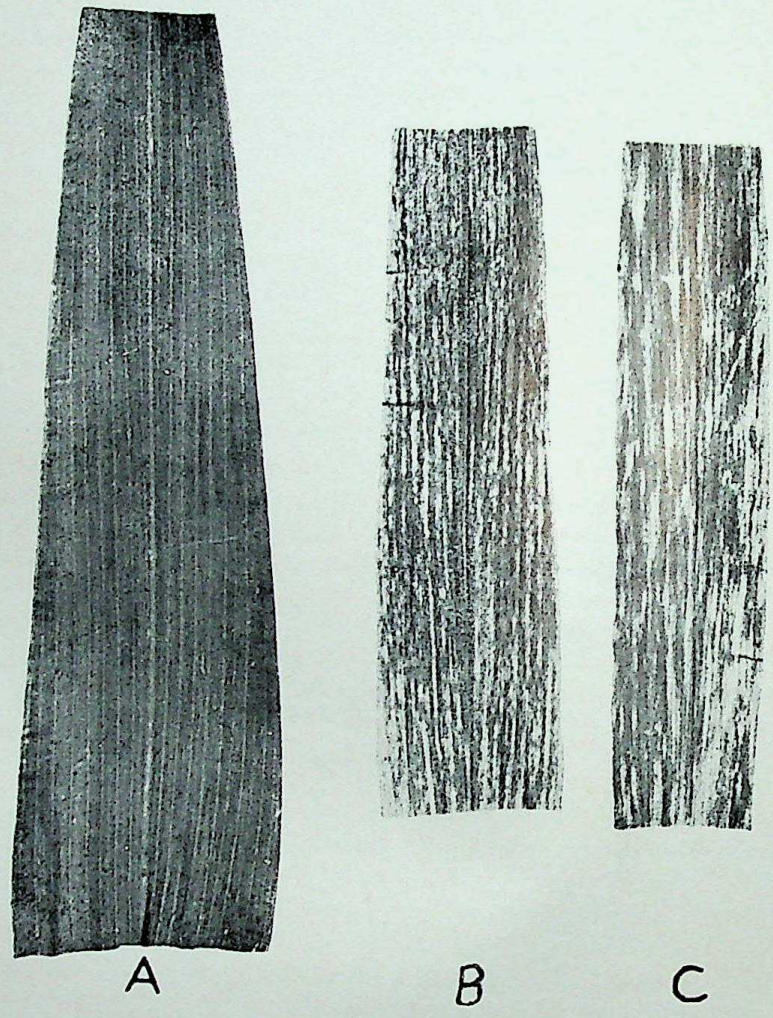


Fig. 2. (A) Healthy leaf, and (B) and (C) leaves of *Zea mays* showing different patterns of mosaic symptoms produced by *Eleusine* mosaic and maize mosaic viruses, respectively.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The soil round these plants was sufficiently loosened and then their roots exposed gently in order to liberate the apterous adult females on them. The aphids usually settled on the exposed roots within a short time. These were again reset and lightly covered with soil. It was observed that the aphids bred fast, and after about 7 to 10 days sufficient number of apterae were available in these colonies.

The test seedlings were also similarly removed from the soil, and their roots were washed with sterile water and kept immersed in water in a dish. Thereafter, a hole about 3-inch deep and 1-inch in diameter was excavated into the soil in a 6-inch clay pot, in which the test seedling was planted after liberation of the aphids. The aerial portion of the test seedling was allowed to rest on the rim of the pot and the exposed roots directed into the excavated hole. Thereafter the required number of infective aphids were gently pushed on the roots which were later covered gently with loose soil. After allowing the aphids to feed on these plants for 24 to 36 hours, the seedlings were again taken out of the soil, their roots washed with 0.01 per cent Folidol in order to kill all aphids on them and then reset in a new pot and kept under observation. These seedlings were sprayed with 0.01 per cent Folidol and also irrigated with the same insecticide as a precaution. Of the three species of *Eleusine*, only *E. coracana* stood the mechanical manipulation best.

SYMPTOMATOLOGY : The virus induces mosaic symptoms in leaves of all the three species of *Eleusine* tested. The mosaic pattern is very clear and pronounced on young leaves (Fig. 1. A.) and at this stage can be clearly distinguished from that induced by the maize mosaic virus (MMV) in *Eleusine coracana* (Chona and Seth, 1960). The mosaic pattern induced by the *Eleusine* mosaic virus (EMV) in maize consists of more or less elongated chlorotic streaks interspersed with light green areas, while that caused by MMV consists of broken and irregular streaks (Fig. 2).

Diseased plants remain stunted and the ear heads of severely affected plants are malformed. Such plants produce few seeds of smaller size. This reduces the yield considerably.

INCIDENCE OF DISEASE IN NATURE : From 1961 to 1963 the incidence of disease was recorded in 2876 plants of *E. coracana*, out of which 302 or 10.50 per cent were diseased. Out of 394 *E. indica* plants only 33 were diseased, while 17 out of 237 plants of *E. aegyptia* were diseased. In addition plants of *Panicum antidotale* Retz., which grows commonly at Poona, showed 29 per cent infection of EMV. It is remarkable that though the virus has a number of other host plants, the disease has not been noted in nature on any of these except *Eleusine* spp. and *P. antidotale*.

TRANSMISSION OF THE VIRUS :

Seed : Out of several hundreds of plants raised in glasshouse from seed collected from diseased plants, not a single plant was infected, even after 70 days. This showed that EMV is not seed-borne.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Soil :

In all 1000 seedlings of *Eleusine* spp. (raised from healthy stock) were transplanted in pots filled with soil in which diseased plants had been grown for over 4 months, and were kept under observation for 70 days. None of the seedlings developed any symptoms of the disease. The EMV is also not soil-borne.

Sap :

Manual inoculation of *E. coracana*, *E. indica*, and *E. aegyptia* seedlings with crude sap resulted in 10 to 35 per cent infection. But with the help of 300-mesh Carborundum infection increased to 65 to 100 per cent. The symptoms of mosaic take about 7-12 days to manifest following sap inoculation.

Aphids :

For these tests viviparous apterous females were liberated in groups of 30 to 40 insects for 24 hours on healthy test plants. Such experiments were possible only with two aphid species, viz., *Rhopalosiphum maidis* and *Longiunguis sacchari*. For other aphids colonies were raised in the insectary on their respective host plants and inoculations were made by liberating them in groups of 30 to 40 per test seedling. At least 100 to 200 seedlings of each test plant were inoculated through each aphid species. The results of these tests are summarised in Table 1.

TABLE 1. Transmission of Eleusine mosaic virus by aphids

Aphid species	Percentage transmission in test plants of			
	E. coracana	*E. indica*	*E. aegyptia*	*Zea mays*
Rhopalosiphum maidis	90.0	62.5	62.0	80.5
Schizaphis cyperi	89.0	68.5	71.5	72.5
Toxoptera graminum	53-5	54.0	49.0	51.0
Longiunguis sacchari	0.0	0.0	0.0	0.0
Pentalonia nigronervosa	0.0	0.0	0.0	0.0
Tetraneura nigriabdominalis	54.5	61.0	62.5	60.0
Aphis gossypii (Common strain)	12.0	12.0	14.0	15.0
A. gossypii (Colocasia strain)	96.5	85.5	81.0	95.0
A. gossypii (Commelina strain)	54.0	57.0	55.0	62.5
Myzus persicae	20.0	21.0	23.0	20.0
Aphis fabae	0.0	0.0	0.0	0.0

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The root aphid, *Tetraneura nigriabdominalis* 1 B. seems to have a wide host range and was collected locally from the roots of *Dicanthium annulatum* (Forssk.) Blume, *Iseilema laxum* Hack., *I. prostratum* (Linn.) Anderss., *E. coracana*, *E. indica*, *E. aegyptia*, *Andropogon sorghum* Pers., *Setaria italica* Beauv., *S. verticillata* (L.) Beauv., *Urochloa marathensis* var. *Velutina* Henr., *Panicum antidotale*, *P. trypheron* Schult., *Dinebra retroflexa* Panzer, *Coix lachrymajobi* L., *Bothriochloa ischaemum* (L.) Keng., *Ischaemum pilosum* Hack., *Andropogon pumilus* Roxb., and *Aristida funiculata* Trin. and Rupr. Several food hosts of this aphid species have been mentioned by David (1958), but our observations show that it commonly breeds on 14 additional host plants, which have not been previously recorded.

The results in Table 1 show that the virus is transmitted by *R. maidis*, *S. cyperi*, *T. graminum*, *T. nigriabdominalis*, *M. persicae* and *A. gossypii*. Although *R. maidis* gave high percentage of transmission, the *Colocasia strain* of *A. gossypii* produced the highest infection. The *Common strain* of *A. gossypii*, which was collected from dicotyledenous hosts, gave the lowest infection. The role of *A. gossypii* in the spread of the virus in nature cannot, therefore, be ruled out.

NATURE OF TRANSMISSION BY RHOPALOSIPHUM MAIDIS :

In order to determine the persistence of EMV in *R. maidis*, which is the commonest species found on *Eleusine* spp., the undermentioned tests were made :

A group of 30 to 40 viviparous apterous females taken from a colony on diseased *Eleusine coracana* were liberated for 1 hour on a healthy *E. coracana* seedling, and during the next hour transferred to a fresh plant. This was continued for 6 hours at hourly intervals. In five tests performed, the virus was invariably transmitted to the seedling exposed to the feeding of aphids during the first hour only and not in any of the seedlings subsequently fed upon by the aphids (Table 2). These tests conclusively prove that the EMV is transmitted by *R. maidis* in a non-persistent manner.

TABLE 2. Non-persistence of Eleusine mosaic virus in *Rhopalosiphum maidis*

Group	Infection in individual plants inoculated in succession at hourly intervals					
	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.
1	+	—	—	—	nt	nt
2	+	—	—	—	nt	nt
3	+	—	—	—	nt	nt
4	+	—	—	—	—	—
5	+	—	—	—	—	—

(+)=Positive infection
(—)=not infected
nt=not tested

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PHYSICAL PROPERTIES : The thermal inactivation, tolerance to dilution and resistance to ageing of the virus were tested by the usual conventional methods. Inoculum was prepared from juice extracted from leaves of *E. coracana* and all dilutions were made with distilled water. The results showed that the virus remained infective after exposure to a temperature of 55°C for 10 minutes, but was inactivated at 60°C, it tolerated dilution of 1 in 10 but lost infectivity at dilutions of 1 in 15 or over. It also lost its infectivity after storage for 3 hours at room temperature.

Although the physical properties of the maize mosaic virus reported by Chona and Seth (1960) differ from those of EMV, it was considered essential to compare these when the inoculum of the two viruses for these studies was taken from the same plant species. The culture of the MMV was kindly supplied by Dr. B. L. Chona, and it was maintained in *E. coracana* and *Zea mays*. Similarly, the EMV was also maintained in *Zea mays* in addition to *E. coracana* for these studies.

We must mention here our complete failure to transmit the MMV from maize to maize and to *E. coracana* through *Rhopalosiphum maidis*, the three strains of *Aphis gossypii* and also *Myzus persicae*. Of these, *A. gossypii* and *R. maidis* have been reported as vectors of the MMV by Chona and Seth (1960). Under similar experimental conditions these aphid species readily transmitted the EMV both to maize and *Eleusine* spp. both from infected *E. coracana* and maize plants at Poona.

The properties of EMV were essentially the same (thermal inactivation point 60°C., tolerance to dilution 1 in 10, and resistance to ageing 2 hr. 30 minutes), when the treated sap was tested on maize seedlings along with *E. coracana* test plants. Similarly, the properties of MMV were tested both on maize and *E. coracana* test seedlings in juice derived from MMV infected maize and *E. coracana* separately. In these tests it was observed that MMV remained infective after exposure to 50°C but not after 55°C., tolerated dilution of 1 in 100 but not of 1 in 500, and retained infectivity after storage for 16 hours at room temperature but was inactivated after 18 hours. Except for the dilution end point these properties are the same as reported by Chona and Seth (1960).

The results of these studies also show that the properties of these viruses did not change even when the source plant was maize in case of EMV and *E. coracana* in case of MMV.

HOST RANGE : All plant species that were used for study of the host range of the virus were raised from seed and inoculated both by sap and through *Rhopalosiphum maidis*. The plant species which were infected are listed in Table 3. All these developed mosaic symptoms similar to those in *E. coracana* and the disease invariably appeared within 10 days after inoculation. The virus was also recovered in *E. coracana* seedlings from all these plants both by sap inoculation and through *R. maidis*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3. Host range of Eleusine mosaic virus

Plant species	Inoculated by sap	Inoculated through *R. maidis*
	a	*a*
Eleusine aegyptia	49/50	50/50
Eleusine indica	48/50	49/50
Eleusine coracana var. H. 1	49/50	50/50
Sorghum vulgare var. M. 47.3	47/50	49/50
Zea mays	50/50	50/50
Euchlaena mexicana	50/50	46/50
Setaria italica var. H-1, H-2, H. K. 289, 282	11/20	14/20
Setaria tomentosa	5/10	6/10
Setaria viridis	16/20	18/20
Setaria verticillata	6/10	8/10
Oplismenus burmannii	6/10	4/10
Urochloa marathensis var. *Velutina*	11/20	13/20
Echinochloa pyramidalis	12/20	14/20
Panicum miliaceum	9/20	12/20
Panicum maximum	10/20	14/20
Ischaemum pilosum	12/20	16/20
Dinebra retroflexa	13/20	19/20
Paspalum scrobiculatum	17/20	19/20
Digitaria sanguinalis	7/15	12/15
Panicum trypheron	9/15	14/15
Bothriochloa ischaemum	7/15	15/15
Panicum antidotale	9/15	13/15
Elettaria cardamomum	8/20	14/20

a-Numerator denotes plants infected.
Denominator denotes plants inoculated.

The following plant species could not be infected either by sap inoculation or through *R. maidis*: *Saccharum officinarum* var. Co. 213, Co. 313, Co. 419, Co. 527, *Erianthus arundinaceus* (Retz) Jeswiet, *Pennisetum typhoideum* Rich., *Hordeum vulgare*, L., *Avena sativa* L.,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Triticum aestivum L., *Oryza sativa* var. Basmathi, *Pennisetum purpureum*, *Amomum subulatum* Roxb., *Cucumis sativus* L., *Musa cavendishii* Lamb., *Allium cepa* L., *Solanum tuberosum* L., *Datura stramonium* L., *Amorphophallus campanulatus* Blume, *Gladiolus* sp., *Colocasia esculenta* and *Cyamopsis tetragonoloba* Taub.

The host range studies show that EMV is of some economic importance to *S. vulgare*, *Z. mays*, *S. italica*, and *E. cardamomum*, but not to sugarcane, wheat, barley, paddy, oats or pearl millet.

DISCUSSION : The physical properties and insect transmission of *Eleusine* mosaic virus indicate that it is distinct from sugarcane mosaic (Smith, 1957) and maize mosaic viruses (Lawas and Fernandez, 1949; Chona and Seth, 1960), and red stripe disease virus (RSDV) of sorghum and maize (Lovisolo, 1957). The physical properties of these viruses show clear distinction from each other (Table 4). Eleusine mosaic virus has the lowest dilution end point and is also comparatively unstable.

TABLE 4. Physical properties of Eleusine mosaic, sugarcane mosaic and maize mosaic viruses, and sorghum red stripe disease virus.

Virus	Dilution end point	Thermal inactivation point	Longevity *in Vitro*	Author
SMV—'X' strain	1 : 100	65°C	6 hrs.	Chona and
SMV—'Y' strain	1 : 100	55°C	6 hrs,	Rafay (1950)
SMV—'Z' strain	1 : 50	45°C	2 hrs.	
SMV	1 : 100,000	55°-60°C	24 hrs.	Costa and Penteado (1951)
MMV	1 : 10,000	45°-50°C	—	Finley (1954)
MMV	1 : 50 to 1 : 100	50°-55°C	16 hrs.	Chona and Seth (1960)
RSDS & M	1 : 1000	46°-50°C	2 hrs. 40 min. to 4 hrs. 50 min.	Lovisolo (1957)
EMV	1 : 15	60°C	2 hrs.	

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The insect transmission data of these viruses is interesting. Though all the four viruses have *Rhopalosiphum maidis* as the common vector, each has a number of additional aphid vectors as shown below :

1. *Sugarcane mosaic virus* : *Toxoptera graminum*, *Hysteroneura setariae* (Ingram and Summers, 1936, 1938), *H, setariae*, *Carolina cyperi* (Tate and Wadey, 1938; Tate and Vandenberg, 1939), *H. setariae* (Charpentier, 1961), *Schizaphis graminum* (Seth and Chona, 1961).

2. *Maize mosaic virus* : *Aphis gossypii* (Lawas and Fernandez, 1949), *A. gossypii*, *Macrosiphum granarium* (Chona and Seth, 1960).

3. *Red stripe disease virus* : *Myzus persicae* (Lovisolo, 1957; Lovisolo and Acimovic, 1961).

4. *Eleusine mosaic virus* : *Aphis gossypii* (3 strains), *Schizaphis cyperi*, *T. graminum*, *Myzus persicae*, *Tetraneura nigriabdominalis* (Present paper).

Chona and Seth (1960) considered *Rhopalosiphum maidis* to be an inefficient vector of maize mosaic virus (7.5 per cent transmission), while *A. gossypii* with 20.6 per cent and *M. granarium* with 28 per cent transmission were considered more efficient. However, we found that *Eleusine* mosaic virus was transmitted most efficiently by the *Colocasia strain* of *A. gossypii* (96.5 per cent transmission), while the *Common strain* of *A. gossypii* gave only 13 per cent transmission. Chona and Seth (1960) did not include *S. cyperi* and *M. persicae* in their transmission studies with the maize mosaic virus. The red stripe disease of sorghum has also been reported to be transmitted by *M. persicae*, although its natural vector is *R. maidis* (Lovisolo, 1957). This virus is distinguished from *Eleusine* mosaic virus on the basis of its physical properties and the characteristic symptoms it causes in sorghum.

Wellman (1934, 1935) obtained transmission of Celery mosaic virus 1 through the *Commelina strain* of *A. gossypii* to *Cucumis sativus*. Although this strain of *A. gossypii* transmitted EMV to the extent of 54 per cent from *Eleusine* to *Eleusine*, it could not transmit the virus to *Cucumis sativus*.

Further, EMV differs from all the 3 viruses in having a root aphid, *Tetraneura nigriabdominalis*, as a vector. Records of plant viruses transmitted through root aphids are few. Thompson *et al.* (1944) thought that *Pemphigus lactucae* Fitch. may act as vector of lettuce big vein disease virus, while McLean (1962) reported transmission of lettuce mosaic virus by *Pemphigus bursarius* (L.). *Eleusine* mosaic virus is, therefore, the 3rd virus transmitted by a root aphid in the world. It is, however, the first record of a root aphid-borne virus in India.

Lastly, the host range of *Eleusine* mosaic virus shows that it cannot infect sugarcane, while both the sugarcane mosaic and maize mosaic viruses can infect *Eleusine* spp. (Kunkel 1924; McRae and Subramanian, 1928; Chona and Seth, 1960). *Euchlaena mexicana* has been demonstrated to be a host of sugarcane mosaic virus (Mitra, 1936)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and of maize mosaic virus (Chona and Seth, 1960), but we found that this plant can be infected by EMV when 3-to 5-day old seedlings are inoculated. Further, the host range of EMV seems to be more extensive than that of sugarcane mosaic and maize mosaic viruses.

On the basis of these findings, EMV is considered distinct from sugarcane mosaic and maize mosaic viruses and also to the red stripe disease virus of sorghum. It is, therefore, designated as "*Eleusine mosaic virus*".

Summary

A mosaic disease of *Eleusine* naturally infecting *E. aegyptia* (L.), Desf., *E. indica* (L.) Gaertn. and *E. coracana* Gaertn. in Poona was found to be caused by a sap-transmissible virus. The symptoms produced in *Eleusine* spp. consist of elongated chlorotic streaks interspersed with light green patches. The virus is readily transmitted through sap inoculation and by the aphids *Rhopalosiphum maidis* Fitch., *Schizaphis cyperi* (V.D.G.), *Toxoptera graminum* (Rond.), *Tetraneura nigriabdominalis* Sasaki, *Aphis gossypii* Glover (three strains) and *Myzus persicae* (Sulz.). Three aphid species, *Longiunguis sacchari* Zehntner, *Pentalonia nigronervosa* Coq. and *Aphis fabae* Scap., did not transmit the virus. The virus is non-persistent in *R. maidis*.

The virus is inactivated by exposure to heat between 55 and 60°C, its dilution end point is at 1 in 15, and it tolerates ageing upto 2 hours 30 minutes at room temperature. These properties did not change when tested in sap extracted either from *Eleusine coracana* or *Zea mays* using both these plant species as test plants also.

The host range of the virus is extensive and includes *Sorghum vulgare* Pers., *Zea mays* L., *Setaria italica* Beauv., and *Elettaria cardamomum* Maton, but does not infect *Saccharum officinarum* L., *Hordeum vulgare* L., *Avena sativa* L., *Triticum aestivum* L., *Oryza sativa* L. and *Pennisetum typhoideum* Rich. among others.

The virus is considered distinct from the sugarcane mosaic and maize mosaic viruses and is designated *Eleusine mosaic virus*.

Indian Agricultural Research Institute,
Plant Virus Research Laboratory,
Poona-5.

References

Brandes, E. W. (1920) Artificial and insect transmission of sugarcane mosaic. *J. agric. Res.* **19** : 131-138.

Brandes E. W. and P. J. Klaphaak (1923) Cultivated and wild hosts of sugarcane or grass mosaic. *J. agric. Res.* **24** : 247-261.

Charpentier, L. J. (1961) Sugarcane mosaic vector studies in Louisiana. *Sug. Bull. N. Orleans*, **39** : 222-225.

Chona, B. L. and S. A. Rafay (1950) Studies on the sugarcane diseases in India. I. Sugarcane mosaic virus. *Indian J. agric. Sci.* **20** : 39-68.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHONA, B. L. AND M. L. SETH (1958) *Aphis maidis* Fitch. as vector of sugarcane mosaic in India. *Indian J. agric. Sci.* **28** : 257-260.

CHONA, B. L. AND M. L. SETH (1960) A mosaic disease of maize (*Zea mays*) in India. *Indian J. agric. Sci.* **30** : 25-32.

COSTA, A. S. AND M. P. PENTEADO (1951) Corn seedlings as test plants for the sugarcane mosaic virus. *Phytopathology* **41** : 758-763.

DAVID, K. S. (1958) Notes on South Indian aphids. V. Greenideinae, Thelaxinae and Eriosomatinae. *Indian J. Ent.* **20** : 124-129.

FINLEY, F. (1954) A mosaic disease of sweet corn. *Phytopathology* (Abs.) **44** : 488.

INGRAM, J. W. AND E. M. SUMMERS (1936) Transmission of sugarcane mosaic by the rusty plum aphid *Hysteroneura setariae*. *J. agric. Res.* **52** : 879-887.

INGRAM, J. W. AND E. M. SUMMERS (1938) Transmission of sugarcane mosaic by the green bug (*Toxoptera graminum* Rond.). *J. agric. Res.* **56** : 537-540.

*KUNKEL, L. O. (1924) Studies on the mosaic of sugarcane. *Bull. Exp. Sta. Hawaiian Sug. Plant. Assoc. Bot. Sci.* **3** : 115-167.

*LAWAS, O. M. AND W. L. FERNANDEZ (1949) A study of the transmission of corn mosaic and some of the physical properties of its virus. *Philipp. agric.* **82** : 231-238.

*LOVISOLO, O. (1957) Contributo sperimentale alla conscenza ed alla determinazione del virus agente dell' aggressomento striato del Sorgo e di un mosaico del Mais. *Boll. Staz. Pat. Veg. Roma.* **14** : 261-321.

LOVISOLO, O. AND M. ACIMOVIC (1961). Sorghum red stripe disease in Yugoslavia. F.A.O. *Plant. Prot. Bull.* **9** : 99-102.

*MATZ, J. (1933) Artificial transmission of sugarcane mosaic. *J. agric. Res.* **46** : 821-839.

MCLEAN, D. L. (1962) Transmission of lettuce mosaic virus by a new vector, *Pemphigus bursarius*. *J. econ. Ent.* **55** : 580-583.

MCRAE, W. (1929). India: new diseases reported during the year 1928. *Internl. Bull. Pl. Prot.* **3** : 21-33.

MCRAE, W. AND L. S. SUBRAMANIAM (1928) A further note on the mosaic disease of sugarcane. *Agric. Jour. India* **23** : 239-255.

---

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MITRA, M. (1936) Report of the Imperial Mycologist. *Sci. Rep. agric. Res. Inst.* Pusa, **1933-34** : 139-167.

RAWLINS, T. E. AND C. M. TOMPKINS (1936) Studies on the effect of Carborundum as an abrasive in plant virus inoculations. *Phytopathology* **26** : 578-587.

*SEIN, F. (1930) A new mechanical method for artificially transmitting sugarcane mosaic. *J. Dept. agric. Peurto Rico.* **14** : 49-68.

SETH, M. L. AND B. L. CHONA (1961). A note on *Schizaphis graminum* (Rondami) an additional vector of sugarcane mosaic in India. *Indian Phytopath.* **14** : 103-104.

SMITH, K. M. (1957) *A Textbook of Plant Virus Diseases.* Second Edition. J. & A. Churchill Ltd., London.

TATE, H. D. AND S. R. VANDENBERG (1939). Transmission of sugarcane mosaic by aphids. *J. agric. Res.* **59** : 73-79.

TATE, H. D. AND F. M. WADEY (1938) Studies on sugarcane insects in Puerto Rico. *Agric. Notes Mayaguez Exp. Sta. P. R.* **83.**

THOMPSON R. C., S. P. DOOLITTLE, AND F. F. SMITH (1944) Investigations on the transmission of big vein of lettuce. *Phytopathology* **34** : 900-904.

VENKATARAYAN, S. V. (1946) Mosaic disease of Ragi (*Eleusine coracana* Gaertn). *Curr. Sci.* **15** : 258-259.

WELLMAN, F. L. (1934) Identification of Celery Virus 1. *Phytopathology* **24** : 695-725.

WELLMAN, F. L. (1935) The host range of the Southern celery mosaic virus. *Phytopathology* **25** : 377-404.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE UPTAKE AND TRANSLOCATION OF LABELLED FUNGICIDES IN PLANTS

II TRANSLOCATION OF FOLIAR APPLIED S^{35} IN POTATO

DHARAM VIR AND B. S. BAJAJ

(Accepted for publication April 12, 1965)

In modern agriculture it is recognised that the chemical control of plant diseases is of tremendous value in supplementing the poor yields of our crops. In the field of fungicides, the range of materials and mode of research have shown rapid strides during the last few years and spraying schedules have been evolved for a number of crop diseases which cause different types of infections on the foliage and other aerial parts of the plant. Of late, there have been some sporadic reports that some of the fungicides, after foliar application, find their way into the plant system resulting in their translocation and subsequent accumulation in edible parts. Miller[1] observed the translocation of mercury to apple fruits as a result of foliar application of fungicide based on mercury. Recently, Ross and Stewart[2] have also reported accumulation of mercury in apple fruits from foliage application of phenyl mercury acetate against apple scab.

In the light of these reports and the possibility of health hazards, an attempt was made to study and compare the translocation and subsequent accumulation of sulphur in tubers of two potato varieties, *i. e.* Up-to-date and *Phulwa*, as a result of foliar application under similar conditions of experimentation and also to investigate the period for which the residues inside the tubers persisted under storage conditions. The experiments were conducted with this fungicide because the dithiocarbamates, which have assumed considerable importance as potato sprays, are based upon sulphur. Moreover S^{35} being an isotope with relatively longer half life (87.1 days) can be used more conveniently for such a type of investigation where it is required to study the period for which the fungicidal residues accumulated in edible parts of plant persist during storage.

EXPERIMENTAL : The investigations were conducted with the help of labelled elemental sulphur (S^{35}) which was obtained from the Atomic Energy Establishment, Trombay, Bombay. Potato plants of variety Up-to-date and *Phulwa* were grown in pots, the planting being done in the first week of November. After about two months, 4 plants of uniform size were selected from each variety. S^{35} (S. A. 5mc/g) was applied twice at a dosage of 423 μc/ml per plant on the two middle leaves at an interval of 20 days. The top portion of the pots was tightly covered with polyethelene cloth in order to prevent the contamination of soil with radio-active material present on the leaves. The untreated control plants were treated with ordinary sulphur in the same manner and kept separate. Digging up of the tubers from the experimental pots

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

was done in the end of February. The tubers were repeatedly washed with water and the presence of the fungicide in the tubers was determined by direct measurement and autoradiographic technique. In the former case, pulp tissue of the tuber was dried at room temperature, finely ground and 150 mg. of this tissue was placed in the form of a thin layer on an aluminium planchet. The planchets containing the active material was supported by three empty planchets in order to increase the back scattering because S^{35} is a soft beta emitter. The relative quantity of this fungicide in different samples was determined by recording the amount of radioactivity taken up in terms of disintegrations per minute based on 5000 or more counts by means of a Geiger Muller Counter (Phillips Universal Scaler). In the autoradiographic technique, the tubers were cut in small thin slices and dried in between the folds of blotting sheets. These were later placed in contact with an X-ray film for about a week and the film was subsequently developed in X-ray developer. Presence of S^{35} was detected in the tubers by both techniques and this indicated that the fungicide has been translocated and thereby accumulated in the tubers of both the varieties as a result of foliage application. The date on quantitative accumulation were recorded for samples obtained from small sized tubers (10-15 g.) and large sized tubers (30-35 g.) and are given in Table I. It is evident from the Table I that there was greater accumulation of the fungicide in smaller tubers of both the varieties than in the bigger ones, probably because of the higher metabolic activity in such a tissue. It is also apparent that there is an appreciable difference in radioactivity between the comparable samples of the varieties, up-to-date and *Phulwa* in both the categories of tubers, indicating that varieties of crop plants may behave differently with regard to absorption, translocation and subsequent accumulation of fungicides when applied as foliage spray.

TABLE 1 : Showing the relative activity in terms of disintegration per minute of various samples from small and big tubers* of the two potato varieties as a result of foliar application of S^{35}.

Sample No.	Up-to-date			Phulwa		
	Small tubers	Big tubers	Control	Small tubers	Big tubers	Control
1.	253.1	230.0	40.2	156.9	132.4	42.8
2.	252.0	209.2	41.5	162.6	123.0	40.5
3.	264.3	232.6	41.4	150.5	125.9	40.0
4.	251.1	205.7	42.8	160.8	125.3	40.8

*Tubers were classified on weight basis which ranged from 10 to 15 g. in the case of small tubers and 30 to 35 g. in the case of big tubers.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In the subsequent studies, some of the tubers of both the varieties showing accumulation of S^{35} as a result of foliar application were stored at 7°C in a referigerator in order to determine the period for which residual effect of the fungicide persisted. After every fortnight a small tissue of the tubers was taken out, dried at room temperature and the activity of 150 mg. of the finely ground tissue was recorded as in the previous experiments. It was observed that the samples of tubers from *Phulwa* and Up-to-date varieties continued to show radio activity for 5-6 months, indicating thereby that the residual effect of the accumulated fungicide persisted in the tubers stored at low temperature (7°C) for over 5 months.

These results clearly show the accumulation of sulphur in the tubers as a result of foliar application and its persistance in the tubers even in storage. Because of the toxicity hazards of fungicides, it would be essential to study the quantitative accumulation of fungicides in edible parts, in relation to dosages applied, number of foliar applications and the period between different applications especially between the last application and harvest of the crop. Work on these lines is in progress.

SUMMARY

Investigations conducted to study the absorption and translocation of foliar applied S^{35} in potato showed that this fungicide accumulated in the tubers of varieties up-to-date and *Phulwa* which were used in the experiments. It was observed that these fungicidal residues persisted inside the tubers during storage for over five months.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for encouragement and going through the manuscript critically.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

[1]Miller, E. J. *Plant Pathology*.. 5 : 119-121 (1956).

[2]Ross, R. G. and D. K. R. Stewart., *Canad. J. Pl. Sci.*, **42** : 280-285.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

UTILIZATION OF AMINO ACIDS AS SOLE SOURCE OF CARBON AND NITROGEN BY SOME XANTHOMONADS

W. V. KOTASTHANE, A. C. PADHYA AND M. K. PATEL

(Accepted for publication May 20, 1965)

Of all the living things, microorganisms are very exacting as to the sources of carbon and nitrogen for their metabolism. This selectivity, many a time, constitutes the only method available for identification of microorganisms whose morphological and biochemical characteristics lie so close to one another as to preclude their differentiation. Among phytopathogenic bacteria, except for the host specificity, there are hardly any other criteria to work with. Thus, fermentation of carbon compounds (Burkholder, 1932 ; Dowson, 1957) and the utilization of nitrogen from various sources (Burkholder, 1939) had been considered for differentiation in this group. Later an attempt was made (Starr and Weiss 1943) to separate the genus Phytomonas into Pseudomonas which utilize aspargine as an only source of carbon and nitrogen and Xanthomonas for which aspargine is not sufficient as an only source of carbon and nitrogen. Since Xanthomonads constitute an economic group amongst the phytopathogenic bacteria, many workers have paid attention to the utilization of amino acids and consensus of results is that, apart from glutamic acid, no other amino acid can serve as a sole source of carbon (Lewis, 1930 ; McNew, 1933 ; Bhide, 1948 ; Kulkarni *et al.* 1952 ; Patel *et al.*, 1949; 1951a; 1951b). We have tried to extend this aspect of utilization of amino acids, *viz.* as a sole source of carbon or of nitrogen by including in this study, 16 Xanthomonads recently isolated in India.

AMINO ACIDS : Twenty amino acids were tested either as a sole source of carbon or of nitrogen :

BASAL MEDIUM : For all experimental work, pyrex glassware was used. This was kept in chromic acid solution for 2-3 days, then washed in running water and finally rinsed twice with glass distilled water. Synthetic medium employed was that recommended by Ayers, Rupp and Johnson (S. A. B. 1957) and had the following composition :

(i) $NH_4H_2PO_4$ – 1.0 g. ; (ii) KCl – 0.2 g. ; (iii) $MgSO_4$, $7H_2O$ – 0.2 g. ; (iv) Distilled water – 1000 ml. ; (v) Glucose (added aseptically after sterilisation) – 5 g. The pH was 7.0.

This medium contains 0.012 per cent nitrogen and 0.04 per cent carbon. The quantity of an amino acid used to replace either carbon or nitrogen of the said medium was equivalent to this percentage ; the actual quantities of various amino acids corresponding to these percentages are given in Table 1. Whenever $NH_4H_2PO_4$ was replaced by an amino acid as the sole source of N in the synthetic medium, deficiency of the phosphate radical was made good by addition of an equivalent amount of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

potassium basic and acid phosphates while the pH was still maintained at 7.0. While testing an amino acid as a sole C source, glucose was omitted from the synthetic medium.

TABLE 1

Amino Acids	Amount in mg. corresponding to	
	0.012 per cent N.	0.04 per cent C.
Glycine	64	625
DL-Alanine	76	494
DL-Serine	90	583
DL-nor-Leucine	112	360
DL-Threonine	102	100
L-Leucine	112	360
DL-Valine	100	390
DL-iso-Leucine	112	360
DL-Aspartic acid	114	550
L-Glutamic acid	126	490
L-Arginine	31	480
DL-Lysine	66	400
L-Asparagine	56	730
L-Histidine	44	430
L-Proline	99	470
L-Hydroxyproline	112	545
L-Tryptophane	87	300
L-Tyrosine	166	330
DL-Methionine	128	496
L-Cystine	103	660

INOCULUM : A 38-48 hours growth on potato dextrose-agar (PDA) at 10°—15°C. (to minimise death rate of cells) was harvested by scraping. After careful washing of the cells by centrifugation, a suspension was prepared in buffer solution (pH 7.0) and was adjusted to contain 10^6—10^8 cells/ml. This suspension was used for inoculation of the medium at the rate of 0.1 ml./5 ml. in test tube. The inoculated medium was then incubated at 28°C.

The visual observations of still cultures made after an incubation period of 96 hours are recorded in tables 2 and 3. Proteose peptone in N series and glucose in C series were used as controls ; checks for sterility of the medium were also carried out simultaneously.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2 : *Utilisation of amino acids as a sole source of Nitrogen**

Xanthomonas	Glycine	DL-Alanine	DL-Serine	DL-nor-Leucine	DL-Threonine	L-Leucine	DL-Valine	DL-iso-Leucine	DL-Aspartic Acid	L-Glutamic acid	L-Arginine	DL-Lysine	L-Asparagine	L-Histidine	L-Proline	L-Hydroxyproline	L-Tryptophane	L-Tyrosine	DL-Methionine	L-Cystine	Proteose-peptone
alangi	2	3	0	0	2	4	4	4	4	4	4	2	4	3	3	4	2	0	4	3	4
bauhiniae	0	3	0	0	3	4	3	3	4	4	2	3	4	3	3	3	2	0	3	4	4
citri	4	4	0	0	4	4	2	0	4	4	3	4	4	4	4	4	2	0	4	2	4
corianderi	3	4	0	0	3	3	3	2	3	4	3	3	4	4	4	4	2	0	3	2	4
durantae	2	4	0	0	3	4	2	3	3	4	2	2	4	2	4	3	2	0	3	0	4
ionidi	0	4	0	0	3	3	2	3	4	4	3	3	4	3	2	2	2	0	3	3	4
lantanae	2	3	0	0	3	3	3	4	4	4	2	3	4	3	3	4	2	0	4	4	4
malvacearum	0	3	0	0	2	3	0	4	2	4	3	2	4	3	3	4	0	0	3	3	4
nakatae-corchori	0	4	0	0	4	4	4	4	4	4	3	3	4	4	3	4	2	0	4	4	4
pateli	3	4	0	0	3	3	0	2	3	4	3	3	4	4	3	4	2	0	3	2	4
poinsetticola	2	4	0	0	4	4	4	4	4	4	2	3	4	4	3	4	2	0	4	4	4
spermacoces	3	4	0	0	3	4	3	4	4	4	3	3	4	3	3	4	2	0	3	2	4
tribuli	3	4	0	0	4	4	2	2	3	4	4	2	4	4	4	4	2	0	3	2	4
thirumalachari	2	2	0	0	3	4	2	2	4	4	2	2	4	4	3	2	2	0	3	3	4
vesicatoria	0	2	0	0	2	0	0	3	4	4	0	0	4	4	3	3	0	0	3	3	4
vitis-trifoliae	0	3	0	0	3	4	3	3	4	4	2	3	4	4	2	2	2	0	3	4	4

*Symbols :—4=Excellent growth ; 3=Good growth ; 2=Fair growth ; 1=Poor growth and 0=No growth.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 3 : *Utilisation of amino acids as a sole source of Carbon.*

Xanthomonas	Glycine	DL-Alanine	DL-Serine	DL-nor-Leucine	DL-Threonine	L-Leucine	DL-Valine	DL-iso-Leucine	DL-Aspartic acid	L-Glutamic acid	L-Arginine	DL-Lysine	L-Asparagine	L-Histidine	L-Proline	L-Hydroxyproline	L-Tryptophane	L-Tyrosine	DL-Methionine	L-Cystine	Basal medium	Basal medium without glucose
alangi	0	3				0				3		0			3	2			0		4	0
bauhiniae	0	3				0				3		0			3	3			0		4	0
citri	0	4				0				4		0			3	0			0		4	0
coriandeli	0	4				0				3		0			3	2			0		4	0
durantae	0	3				0				2		0			3	3			0		4	0
ionidi	0	3				0				3		0			3	3			0		4	0
lantanae	0	4				0				3		0			3	3			0		4	0
malvacearum	0	2				0				3		0			2	2			0		4	0
nakatae-corchori	0	3				0				3		0			3	3			0		4	0
pateli	0	3				0				2		0			3	0			0		4	0
poinsetticola	0	4				0				3		0			3	3			0		4	0
spermacoces	0	4				0				3		0			3	3			0		4	0
tribuli	0	4				0				3		0			2	3			0		4	0
thirumalachari	0	3				0				3		0			3	2			0		4	0
vesicatoria	0	3				0				3		0			3	3			0		4	0
vitis-trifoliae	0	2				0				3		0			3	3			0		4	0

*Symbols :—4=Excellent growth ; 3=Good growth ; 2=Fair growth ; 1=Poor growth and 0=No growth.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Analysis of Results

(a) Amino acids as a sole source of Carbon :

DL-Alanine, L-Proline and L-Glutamic acid can supply carbon to all the organisms. L-Hydroxy-Proline can do so for all except for *X. citri* and *X. pateli*. Other amino acids do not serve the purpose.

(b) Amino acids as a sole source of Nitrogen :

DL-nor-Leucine, DL-Serine, and L-Tyrosine do not supply nitrogen and incidentally carbon too. Other amino acids with the following notable exceptions, can serve the purpose as a sole source of nitrogen :

X. vesicatoria does not utilise DL-Lysine, L-Arginine, DL-Valine, L-Tryptophane, Glycine and L-Leucine.

X. malvacearum does not utilise DL-Valine, L-Tryptophane and Glycine.

X.pateli does not utilise DL-Valine.

X.citri does not utilise DL-iso-Leucine.

X.durantae does not utilise L-Cystine.

X.bauhiniae, *X.vitis-trifoliae*, *X.nakatae-corchori* and *X.ionidi* do not utilise Glycine.

DISCUSSION : Data on the utilisation of amino acids by *Xanthomonas* species available in literature has been at variance. The probable cause may be the use of, either non-uniform synthetic medium, or indifferent size of inoculum or unstandardised amount of amino acids. These results have been further vitiated by the fact that the amount of N already present in the synthetic medium, *i. e.* before addition of amino acid has not been taken into account by several workers. This is rather significant, since Xanthomonads are known to grow with inorganic source of nitrogen alone. In the present studies, the amounts of amino acids added have been adjusted to confirm with the carbon and nitrogen content of Ayers, Rupp and Johnson's synthetic medium on which all the 16 Xanthomonads grow luxuriantly. The present investigation should give a comparative idea regarding the utilisation of these elements from amino acids. These studies also reveal that the addition of phosphates in the medium is imperative.

The medium used in this investigation is simple and efficient as compared to several complex media employed by other workers.

This limited study shows some striking differences in the utilisation of nitrogen and carbon of amino acids by Xanthomonads. These may be taken advantage of in their identification along with routine host specificity.

SUMMARY

Data on the utilisation of amino acids as a sole source of carbon or nitrogen by *Xanthomonas* spp. has been presented. DL-Alanine,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

L-Proline and L-Glutamic acid can serve as a source for carbon or nitrogen, while DL-nor-Leucine, DL-Serine and L-Tyrosine can neither supply carbon nor nitrogen. An attempt has been made to arrive at a standard routine procedure for such studies.

We express our deep sense of gratitude to Drs. M. J. Thirumalachar and M. V. Nayudu for helpful suggestions.

Department of Microbiology,
Garda College and Baria Science Institute,
Navsari (Gujarat State)

References

BHIDE V. P. (1948) A comparative study of some wilt producing phytopathogenic bacteria. *Indian Phytopath.* **1** : 79-91.

BURKHOLDER, W. H. (1932) Carbohydrate fermentation by certain closely related species in the genus *Phytomonas*. *Phytopathology* **22** : 554-560.

————(1939) The taxonomy and nomenclature of the phytopathogenic bacteria. *Phytopathology* **29** : 122-139.

DOWSON W. J. (1957) Mannual of bacterial plant diseases. 2nd Ed. Adams and Charles Black, London.

KULKARNI Y. S., M. K. PATEL AND G. W. DHANDE (1952) Bacterial leaf-spot of *Cassia tora*. *Indian Phytopath.* **5** : 76-88.

————(1952) A new bacterial leaf-spot and stem canker of pigeon pea. *Indian Phytopath.* **5** : 21-22.

LEWIS I. M. (1930) Growth of plant pathogenic bacteria in synthetic culture media with special reference to *Phytomonas malvaceara*. *Phytopathology* **20** : 723-731.

MCNEW G. L. (1938) The relation of nitrogen nutrition to virulence in *Phytomonas stewarti*. *Phytopathology*, **28** : 769-787.

PATEL M. K. AND Y. S. KULKARNI (1949) Nitrogen utilisation by *Xanthomonas malvacearum* (Sm.) Dowson. *Indian Phytopath.* **2** : 62-64.

————G. W. DHANDE AND Y. S. KULKARNI (1951) Studies in some species of *Xanthomonas*. *Indian Phytopath.* **4** : 122-140.

————V. V. BHATT AND Y. S. KULKARNI (1951) Three new bacterial diseases of plants. *Indian Phytopath.* **4** : 144-151.

STARK M. P. AND J. S. WEISS (1943) Growth of phytopathogenic bacteria in the synthetic asparagine medium. *Phytopathology* **33** : 314-318.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FACTORS AFFECTING CONIDIAL PRODUCTION OF HELMINTHOSPORIUM ORYZAE

S. B. CHATTOPADHYAY AND C. DAS GUPTA

(Accepted for publication May 29, 1965)

INTRODUCTION : Conidial production of *Helminthosporium oryzae* Breda de Haan on artificial media has been observed to be unsatisfactory as compared with other species of Helminthosporium (Akai and Tanaka, 1954, Mitra 1931). Effect of different factors on conidial production of *H. oryzae* has not been studied extensively, although this important pathogen of rice plant has been a subject of investigation for many years. The foresaid paper gives a summarized account of the investigation on the effect of different media, temperature, relative humidity, carbohydrate and nitrogen sources and their relative concentration on conidial production of *H. oryzae*. This information is considered to be pertinent and basic to an understanding of epidemiology of this disease of rice.

MATERIALS AND METHODS : Eight isolates of *H. oryzae* were used in the present study. The media used for study of conidial production were Richard's synthetic agar, Potato dextrose agar, Oat meal agar, Cornmeal agar and Rice Agar.

Composition of oat, Corn or rice meal agar were :

Oat Corn or rice meal	60 grams.
agar	20 grams.
Water	1000 ml.

Crushed maize, chopped straw or chopped green leaves were also used in the study (25 gms. of crushed maize or green leaves or straw in 100 ml. of water in 250 ml. Conical flask).

For study of effect of different carbon compounds, starch, glucose and sucrose were used, the carbon concentration of starch and glucose was kept equivalent to that of six per cent sucrose.

Asparagin, peptone, ammonium sulphate, ammonium nitrate, alanine, tyrosine, tryptophane, glutamic acid and potassium nitrate were used as different sources of nitrogen, the nitrogen concentration in each case being kept as equivalent to 0.2 per cent asparagin. The media in each case (excepting media of crushed maize or chopped straw or green leaves) was adjusted to pH 6.4 which was found to be optimum for growth.

[1]Present address : Professor of Plant Pathology and Principal, College of Agriculture, University of Kalyani, West Bengal.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Petriplates of 10 cm. diameter containing 25 ml. of the media were used, except in the cases when crushed corn, and chopped green leaves, when 250 ml. Erlenmeyer flasks containing the media as described before were taken. In each case the media were inoculated with 4 days old mycelial growth on potato dextrose agar at 26° C.

The intensity of conidial production was determined microscopically after 14 days' growth and was expressed symbolically as follows :

Nil-n, trace-t, poor-x, moderate-xx,good-xxx, very good-xxxx.

The effect of relative humidity on conidial production was determined at 26° C by exposing petriplates containing Richard's synthetic agar after, 3 days' growth of the fungus, which were kept inside dishes containing mixture of sulphuric acid and water to maintain the requisite relative humidity (Mclean and Cooke. 1941).

EXPERIMENTAL RESULTS : Data on the effect of media, temperature relative humidity, different sources of carbohydrate and nitrogen and varying leaves of carbohydrate and nitrogen on conidial production of *H. oryzae* are presented in Tables 1-6.

TABLE 1 : Conidial production of 8 isolates of H. oryzae in different media at 26° C.

Isolate	Richard's agar	Oatmeal agar	Cornmeal agar	Ricemeal agar	Potato dextrose agar	N/2 potato dextrose agar	2 per cent Water agar	Crushed corn	Chopped straw of rice	Chopped green leaves of rice
H_2	n-x	n-xx	n-xx	n-xx	n-x	n	x	n-xx	n-t	n-xx
H_4	xxx	xxx	xxx	xxx	xx	xx	xx	xxx	xx	xxx
H_6	n-t	n-x	n-x	n-x	n	n	t	n-t	n-t	x
H_7	xxx	xxxx	xxxx	xxxx	xxx	xx	xx	xxx	xxx	xxx
H_{10}	xxx	xxxx	xxxx	xxx	xx	xx	xx	xxx	xxx	xxx
H_{21}	n-x	n-xx	n-xx	n-xx	n-x	n-x	n-x	n-xx	xx	xx
H_{23}	xxx	xxxx	xxx	xxx	xx	xx	xx	xxx	xxx	xxx
H_{27}	n-x	n-x	n-xx	n-xx	n-t	n-t	t	n-xx	xx	xx

Media (Table 1). Media rich in plant parts, namely Oat meal agar, Corn meal agar and crushed corn are more favourable for conidial production, particularly in respect of the isolates which are sparely sporulating Sterilized chopped green leaves in a few cases has been found to be suitable substrates for sporulation. Mitra (3) in connection with studies on

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the effect of media on sporulation of *H. oryzae* noted that the conidial production of *H. oryzae* more intense on chopped straw, as compared to others.

Temperature (*Table 2*)

Temperature of 21°—26° C is optimum for conidial production. Higher temperature inhibits conidial formation ; at 31° C, sporulation is comparatively poor and at 36° C it is absent in sparsely sporulating isolates and poor in heavily sporulating ones.

TABLE 2 : Effect of temperature on conidia production of 8 isolates of *H. oryzae*, on Richard's Agar

Isolates	Temperature			
	20° C	26° C	31° C	36° C
H_1	n-t	n-xx	n-x	n
H_4	xxx	xxx	xx	x
H_6	n-t	n-t	n	n
H_7	xxx	xxxx	xx	x
H_{10}	xx	xxx	xx	x
H_{21}	n-t	n-x	n-t	n
H_{23}	xxx	xxx	xx	x
H_{27}	n-x	n-x	n-t	n

TABLE 3 : Effect of relative humidity on conidia production of 8 isolates of *H. oryzae* at 26° C on Richard's Synthetic agar

Isolate	Relative humidity						
	75.6 per cent	82.9 per cent	88.5 per cent	92.9 per cent	96.1 per cent	98.5 per cent	100 per cent
H_2	n-t	n-t	n-t	n-x	n-xx	n-xx	n-xxx
H_4	xx	xx	xxx	xxx	xxx	xxxx	xxxx
H_6	n	n	n	n-t	n-x	n-x	n-xx
H_7	xxx	xxx	xxx	xxxx	xxxx	xxxx	xxxx
H_{10}	xx	xx	xx	xx	xxx	xxx	xxxx
H_{21}	n-t	n-t	[illegible]	n-x	n-x	n-xx	n-xxx
H_{23}	xxx	xxx	xxx	xxxx	xxxx	xxxx	xxxx
H_{27}	n-t	n-t	n-t	n-x	n-x	n-x	n-xx

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Relative Humidity (Table 3)

Sparsely sporulating isolates require minimum relative humidity of 92.9 per cent for conidial production which increase with further increase in relative humidity, the optimum level being 100 per cent.

Heavily sporulating ones show formation of conidia even at 75.6 per cent relative humidity ; but intensity of sporulation is increased with increase in relative humidity. Relatively Humidity of 96.1—100 per cent has been found to be optimum for conidial production in these isolates.

Different sources of carbohydrates (Table 4)

Conidial production has been observed to be the best in Richard's medium containing either both sucrose and starch or containing six per cent starch alone. In medium containing only sucrose, conidial production of the sparsely sporulating isolates has been practically inhibited. Glucose is a poor source of carbohydrate for conidial production. In medium containing glucose, conidial production of heavily sporulating isolates is very adversely affected, and the sparsely sporulating ones fail to produce any conidia.

TABLE 4 : Conidia production of 8 isolates of *H. oryzae* in Richard's agar with different sources of carbohydrates at 26° C

Isolate	Richard's medium			
	Normal	6 per cent Starch	6 per cent Sucrose	6 per cent Glucose
H_2	n-x	n-x	n-t	n
H_4	xxx	xxx	xxx	x
H_6	n-t	n-t	n	n
H_7	xxx	xxx	xxx	xx
H_{10}	xxx	xxx	xx	x
H_{21}	xxx	xxx	xxx	xx
H_{23}	n-x	n-x	n-t	n
H_{27}	n-x	n-t	n-t	n

Different sources of nitrogen (Table 5)

Of the different sources of nitrogen used, 1 per cent potassium nitrate, asparagin and glumatic acid have been found to favour conidial production. NH_4-salts, alanine, tyrosine and tryptophane have been observed to be poor sources of nitrogen for conidial production.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 5 : Data on effect of different sources of nitrogen on conidia production of 8 isolates of H. *oryzae* at 26°C.

Isolate.	Richard's medium with								
	1 per cent KNO_3	0.2 per cent Asparagin	1 per cent Peptone	0.2 per cent $(NH_4)_2 SO_4$	0.12 per cent $NN_4 NO_3$	0.2 per cent Alanine	0.54 per cent Tyrosine	0.3 per cent Tryptophane	0.44 per cent Glutamic acid
H_2	n-x	n-x	n-x	n	n	n	n-x	n	n-t
H_4	xxx	xxx	xxx	x	x	xx	xx	x	xxx
H_6	n-t	n	n-x	n	n	n	n-t	n	n-t
H_7	xxx	xxx	xxxx	xx	xx	xx	xx	x	xxx
H_{10}	xxx	xx	xx	x	xx	xx	xx	x	xxx
H_{21}	n-x	n-x	n-xx	n	n	n-t	n-x	n	n-x
H_{23}	xxx	xxx	xxx	xx	xx	xx	xx	xx	xxx
H_{27}	n-x	n-t	n-x	n	n	n	n-t	n	n-t

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 6 : Data on effect of different levels of sucrose and KNO_3 in Richard's medium on conidia production of 8 isolates of H. *oryzae* at 26°C.

	Richard's medium with different levels of sucrose and KNO_3											
	5 per cent Sucrose and 1 per cent KNO_3	4 per cent Sucrose and 1 per cent KNO_3	3 per cent Sucrose and 1 per cent KNO_3	2 per cent Sucrose and 1 per cent KNO_3	1 per cent Sucrose and 1 per cent KNO_3	0.5 per cent Sucrose and 1 per cent KNO_3	5 per cent Sucrose and 2 per cent KNO_3	5 per cent Sucrose and 0.8 per cent KNO_3	5 per cent Sucrose and 0.6 per cent KNO_3	5 per cent Sucrose and 0.5 per cent KNO_3	5 per cent Sucrose and 0.4 per cent KNO_3	5 per cent Sucrose and 0.2 per cent KNO_3
H^2	n-x	n-t	n-t	n-t	n-t	n-t	n-x	n-t	n	n	n	n
H^4	xxx	xxx	xxx	xxx	xx	xxx	xxx	xxx	xx	xx	x	x
H^6	n-t	n-t	n-t	n-t	n	n	n-t	n	n	n	n	n
H^7	xxxx	xxxx	xxxx	xxxx	xxx	xxx	xxxx	xxx	xx	xx	x	x
H_{10}	xxx	xxx	xxx	xxx	xx	xx	xxx	xx	xx	x	x	x
H_{21}	n-x	n-x	n-x	n-x	n-x	n-x	n-x	n	n	n	n	n
H_{23}	xxxx	xxxx	xxx	xxx	xxx	xxx	xxxx	xxx	xx	xx	x	x
H_{27}	n-x	n-t	n-t	n-t	n-t	n-t	n-t	n-t	n	n	n	n

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Different levels of carbohydrate (Sucrose) and nitrogen (Potassium nitrate) (Table 6).

Reduction in sucrose content does not materially affect conidial production until a very low level (1 per cent to 0.5 per cent) is reached under which condition sporulation tends to be slightly adversely affected. On the other hand, decrease in potassium nitrate concentration adversely affects sporulation which is marked even at 0.8 per cent level (1 per cent normal). In sparsely sporulating isolates conidial production is practically inhibited at the level. In respect of heavily sporulating ones, the conidial production decreases with decrease in potassium nitrate concentration but is not completely inhibited.

DISCUSSION : Study of sporulation of the isolates of *H. oryzae* under different physiological conditions shows that the isolates can be grouped into sparsely sporulating and heavily sporulating types. Heavily sporulating types readily form conidia under all conditions, while sparsely sporulating ones only under specific conditions. Conidia formation may be an intrinsic character of the isolate. Isolates do not appear to differ from one another in requirements of nutrition. Different carbohydrate and nitrogenous compounds have differential effect on sporulation. Good sporulation of fungus when nitrogen is supplied in the form of KNO_3 shows that the fungus can synthesize all organic nitrogenous substances from inorganic sources. Reduction or suppression of sporulation with decreasing level of potassium nitrate as compared with sucrose, shows that conidial production is more dependent on nitrogen and favourable production of conidia takes place within a narrow optium range.

SUMMARY

Conidial production of *Helminthosporium oryzae* Breda de Haan is favoured by media either rich in plant parts or exclusively of plant parts. Temperature 21-26°C is optimum for their products.

Relative humidity of 92.9 per cent and above favours production of conidia with maximum production at 100 per cent.

Richard's medium containing both starch and sucrose or containing starch is better for growth and sporulation than with sucrose. Glucose is a poor source of carbohydrate for the purpose.

Conidial production is better with KNO_3, peptone, glutamic acid and asparagin as sources of nitrogen in comparison with NH_4 salts, alanine, tyrosine or tryptophane.

Decrease in concentration of sucrose and KNO_3 in Richard's medium shows that reduction in sucrose content does not affect conidial formation much in concentration. Decrease in concentration of KNO_3 adversely affects the conidial production in heavily sporulating isolates and completely inhibits the same in sparsely sporulating ones.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The authors express their sincere gratitude to late Prof. J. G. Dickson, University of Wisconsin, U. S. A. for help and suggestions in the preparation of this manuscript.

College of Agriculture,
University of Kalyani,
West Bengal and
State Agricultural Research Institute,
Calcutta-40.

[1]Akai, S. and H. Tanaka *Memoirs of College of Agric. Kyoto University*, 69 (1954).

[2]Mclean, R. C. and W. R. Cook *Plant science formulae.* Oliver and Boyd. London (1941).

[3]Mitra, M. *Trans. Brit. mycol. Soc.* **15** : 254-293 (1931).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE PATHOGENICITY AND HOST RANGE OF THREE SPECIES OF ROOT-KNOT NEMATODES

A. CHIDAMBARANATHAN AND G. RANGASWAMI

(Accepted for publication May 29, 1965)

In an earlier report from this laboratory the host-range and histopathology of the root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood, was reported (Balasubramanian and Rangaswami, 1964). This nematode species, first reported to be pathogenic to sugarcane in South India (Rangaswami *et al.* 1960), has since been found to infect on artificial inoculation, several plant species (Rangaswami *et al.*, 1961, Balasubramanian and Rangaswami, 1964). With a view to examine the natural occurrence of this and other species of nematode on the vegetable crops of the tract, studies were made by the authors during 1962-63 when three species of *Meloidogyne* occurring respectively on brinjal, chilli and tomato were commonly met with. These three species were examined in detail for their life cycle, host specificity, etc., following the standard procedures, and the nematodes were identified as follows: *Meloidogyne arenaria* Chitwood on eggplant, *M. javanica* (Treub) Chitwood on tomato and *M. incognita* Chitwood on chilli.

MATERIALS AND METHODS : The effect of nematode larvae on the seedlings of host plants was studied by the following procedure. Seeds of the variety Long Purple in eggplant (*Solanum melongena* L.), Country Best in chilli (*Capsicum annum* L.) and Marglobe in tomato (*Lycopersicon esculentum* Mill.) were surface sterilized with $NaOCl_2$ at 1 per cent level of Cl_2 in water, and sown in sterilized soil contained in zinc trays. Freshly collected egg masses were placed in distilled water in clean watch glasses and incubated for 24 hours. The larvae hatching out of the eggs were separated and used for inoculating the seedlings. The seedlings raised in sterile soil in zinc trays, mentioned above, were pulled out, the roots washed free of soil and placed in the watch glass containing the nematode larvae. Five seedlings were placed in each watch glass, in such a manner to immerse the roots only under water with the larval suspension. Then the roots were covered with a thin layer of sterilized fine sand. The dishes were incubated in darkness for 24 hours after which the seedlings were removed, washed free of nematodes and planted in germination boxes containing sterilized soil. The nematode growth inside the root was studied following the methods of Raski (1950) and Triantaphyllou and Hirschmann (1960). The growth rate of the nematodes was studied by measuring the cross sectional area of the nematode and interpreting the sequential data graphically (Ward, 1959).

EXPERIMENTAL RESULTS : *Effect of the nematodes on seed germination and seedling growth* : A comparison of the effects of the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : A comparison of the effect of nematode larvae on seed germination and post emergence seedling growth.
(Data represent average of 4 replications).

Treatment	No. of seeds sown	No. of seedlings survived	Shoot characters			Root characters				
			Average height in cm.	Average No. of leaves	Average dry wt. of shoots in g.	Average root length in cm.	Average No. of lateral roots	Average No. of galls.	Average No. of egg masses per plant	Average wet wt. of roots in g.
Eggplant :										
Check	25	19.5	7.4	3.2	0.101	8.5	23.9	—	—	0.14
Inoculated with *M. arenaraia*	25	19.0	6.5	3.3	0.063	5.8	15.3	6.3	12.8	0.09
Per cent reduction	—	—	12	—	38	34	36	—	—	36
Chilli :										
Check	25	20.8	8.0	4.6	0.073	6.3	15.5	—	—	0.125
Inoculated with *M. incognita*	25	20.0	6.3	4.5	0.053	5.0	10.6	7.3	12.5	0.089
Per cent reduction	—	—	21	—	27	21	32	—	—	29
Tomato :										
Check	25	20.0	12.0	4.8	0.128	6.7	26.4	—	—	0.169
Inoculated with *M. javanica*	25	11.8	10.8	4.7	0.106	3.9	14.9	7.6	8.8	0.150
Per cent reduction	—	41	10	2	17	42	44	—	—	11

Digitized by Arya Samaj Foundation Chennai and eGangotri
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

nematodes on seed germination and post-emergence growth of the seedlings of the respective hosts is made in Table 1. *M. javanica* caused a considerable reduction in seed germination and post-emergence stand of tomato seedlings, whereas the other two species did not materially affect neither the germination nor stand. The nematodes, in general, stunted the roots and shoots, reduced the weights of shoots and roots and also the average number of lateral roots.

(ii) *Cross inoculation studies* : The results on the cross inoculation studies with the three nematode species are summarized in Table 2. *M. arenaria* could infect only eggplant and tomato but not the chilli roots. Growth of this parasite seemed to be quicker in eggplant, than in tomato, as it could attain sexual maturity in 24 days in the former as against 26 days in the latter. Also, the nematode attained a bigger size in eggplant roots than in tomato. *M. incognita* could infect eggplant, chilli and tomato and successfully complete its life cycle. Its growth was quicker in chilli followed by eggplant and tomato, and also the maximum size of the nematode in the three hosts was of the same order. The growth rate of the parasite showed apparent variations from the 12th day onwards ; while it emerged and grew faster from the 12th day in eggplant and 18th day in chilli, it continued in the sausage form in tomato roots until the 24th day. *M. javanica* could infect eggplant, chilli and tomato, but could successfully complete its life cycle only in tomato and also in eggplant, but not in chilli. Though the larvae penetrated chilli roots and were active for a few days, their activity ceased after the sixth day. The nematode grew faster and moulted earlier in eggplant than in tomato, but in the latter it grew to a bigger size and emerged out only on the 26th day.

TABLE 2 : Growth of three root-knot nematodes in the three host plants.

Host plant	Root-knot nematode species					
	M. arenaria		*M. incognita*		*M. javanica*	
	Maximum size attained (1000 sq. microns)	No. of days taken for maturity and egg laying	Maximum size attained (1000 sq. microns)	No. of days taken for maturity and egg laying	Maximum size attained (1000 sq. microns)	No. of days taken for maturity and egg laying
Eggplant	244	24	133	3	169	28
Chilli	No infection		[illegible]	22	No infection	
Tomato	173	26	98	34	204	30

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(iii) *Host ranges of the three nematodes* : A comparison of the host ranges of the three nematode species, carried out simultaneously under identical conditions, is made in Table 3. Of the 8 plant species tested, only clusterbeans (*Cyamopsis tetragonoloba* Taub.) was resistant to all the three species. *Solanum tarvum* Swartz was resistant to *M. arenaria* and *M. javanica*, but was highly susceptible to *M. incognita*. The other six hosts varied in their susceptibility to the three nematode species. In general, *M. javanica* was found to produce the maximum sized galls in the tap roots of susceptible hosts, *M. arenaria* coming next, produced globular galls mostly on the lateral roots, and *M. incognita* caused only small galls at the basal portions of lateral roots.

TABLE 3 : Studies on the host range for the three nematode species. Results expressed as an index* of root-knot caused on different hosts.

(Data based on 9 plants inoculated under each nematode and host species : final readings taken 45 days after inoculation).

Nematode species	*Hibiscus esculentus*	*Vigna catjang*	*Dolichos lablab*	*Cyamopsis tetragonaloba*	*Solanum nigrum*	*Solanum tarvum*	*Physalis minima*	*Nicotiana tabacum*
M. arenaria	1	3	1	0	3	0	3	4
M. incognita	3	3	2	0	1	4	2	3
M. javanica	4	1	3	0	2	0	4	4

*As per the method of Winstead and Sasser 1956.

DISCUSSION : The life cycles of the three nematode species studied were similar to the one described by Chitwood (1951).

Studies on the effect of the three nematode species on the seed germination revealed, that only with *M. javanica* there is considerable reductions in the germination percentage, while in the others there was no effect. Severe mortality in galled seedlings due to nematode infections was reported by Steiner (1940) and Winstead and Sasser (1956). The adverse effects on the emerging seedlings and their subsequent growth have been brought out by the significant reductions in their shoot and root heights and weights. These results are in line with the reports of Chitwood (1951), Whitehead (1959) and Chapman (1960).

In the cross inoculation studies *M. incognita* from chilli was found infective to chilli, eggplant and tomato, *M. arenaria* from eggplant and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

M. javanica from tomato infective on only eggplant and tomato, but not chilli. There were also marked differences in the size of nematodes and the number of days taken for their maturity in different hosts. In the host range studies tobacco was found highly susceptible and clusterbeans highly resistant to all the three nematodes, the others showing varying responses. Several workers have reported on the influence of environmental conditions on the susceptibility of host plants to nematode infection (Sasser 1954, Minz 1956, Martin 1958). Chilli is reported non-susceptible to *M. arenaria* in U. S. A. by Hare (1956), whereas Sasser (1952) from the same country found it susceptible. Also, Linde (1956) found the plants susceptible to *M. incognita*. In the present studies chilli plants were found susceptible only to *M. incognita* but not to *M. arenaria* and *M. javanica*. Tomato plants were found to be severely affected by all the three species of nematode and this confirms the report of Minz (1956), who found tomato to harbour many species of *Meloidogyne* in Israel and also similar reports by Linde (1956) from South Africa and Martin (1958) from Rhodesia. However, one should realise the influence of various agronomic and climatic conditions on host-parasite relationships, before successful infection symptoms are brought about.

Summary

On the basis of their morphology, pathogenicity and life cycle three root-knot nematode species occurring on three of the important vegetable crops of South India were identified as follows : on egg plant as *Meloidogyne arenaria* Chitwood, on chilli as *M. incognita* Chitwood, and on tomato as *M. javanica* (Treub) Chitwood. *M. javanica* caused a severe reduction in seed germination and stand of tomato seedlings whereas the other two species did not cause any such reductions. All the three species caused reductions in shoot and root lengths and in their weights. In the host range studies, tobacco was found most susceptible to all the three nematode species and clusterbeans most resistant.

Microbiology Laboratory,
Faculty of Agriculture,
Annamalai University,
Annamalainagar, Madras State.

References

Balasubramanian, M. and G. Rangaswami (1964) Studies on host range and histopathology of root-knot infections caused by *Meloidogyne javanica*. *Indian Phytopath.* **17** : 126-132.

Bird, A. F. (1959) Development of the root-knot nematodes, *Meloidogyne javanica* and *M. hapla* Chitwood, in the tomato. *Nematologica* **4** : 31-42.

Chapman, R. A. (1960) The effect of *Meloidogyne incognita* and *M. hapla* on the growth of [illegible] and red clover and Atlantic alfalfa. *Phytopathology* **50** : 181-182.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHITWOOD, M. D. (1951) Notes on the physiology of *Meloidogyne javanica* (Treub, 1885), Nematoda, Heteroderidae. *J. Parasitol.* **31** : 96-98.

HARE, W. W. (1956) Comparative resistance of seven pepper varieties to five root-knot nematodes. *Phytopathology* **46** : 669-672.

LINDE, W. J. (1956) The *Meloidogyne* problem in South Africa. *Nematologica*, **1** : 177-183.

MARTIN, G. C. (1958) Root-knot nematodes, *Meloidogyne* spp., in the Federation of Rhodesia and Nyasaland. *Nematologica* **3** : 332-349.

MINZ, G. (1956) The root-knot nematode, *Meloidogyne* spp. in Israel. *Plant Dis. Reptr.* **40** : 798-801.

RANGASWAMI, G., V. N. VASANTHARAJAN, AND R. VENKATESAN (1960) The occurrence of root-knot nematodes on sugarcane and on some weeds. *Curr. Sci.*, **29** : 236-237.

RANGASWAMI, G., M. BALASUBRAMANIAN AND V. N. VASANTHARAJAN (1961) The host range of sugarcane root-knot nematode *Meloidogyne javanica*. *Curr. Sci.* **30** : 149-150.

RASKI, D. J. (1950) Life history and morphology of the sugar beet nematode *Heterodera schachti*. *Phytopathology* **40** : 135-162.

SASSER, J. N. (1952) Identification of root-knot nematodes *Meloidogyne* spp. by host reactions. *Plant Dis. Reptr.* **36** : 84-86.

————— (1954) Identification and host-parasite relationships of certain root-knot nematodes, *Meloidogyne* spp. *Md. Agr. Expt. Sta. Bull. A. 77.*

STEINER, G. (1940) The root-knot nematodes attacking stems of plants. *Phytopathology* **30** : 710.

TRIANTAPHYLLOU, A. C. AND H. HIRSCHMANN (1960) Post-infection development of *Meloidogyne incognita*. Ann. Inst. Phytopath. *Benaki, N. S.*, **3** : 3-12.

WHITEHEAD, A. G. (1959) The root-knot nematodes of Africa I. *Meloidogyne africana* n. sp., a parasite from arabica coffee. *Nematologica* **4** : 272-278.

WINSTEAD, N. N. AND J. N. SASSER (1956) Reaction of cucumber varieties to five root-knot nematodes, *Meloidogyne* spp. *Plant Dis. Reptr.* **40** : 272-275.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EVALUATION OF HOST SPECIFICITY CHARACTER IN THE DIFFERENTIATION OF XANTHOMONAS SPECIES

M. K. PATEL, M. C. SRINIVASAN AND M. J. THIRUMALACHAR

(Accepted for publication August 17, 1965)

The genus *Xanthomonas* Dowson includes several phytopathogenic bacteria, which show in a general way uniformity in their morphological and physiological characters. They are gram negative rods which are motile by single polar flagella and grow well on sugar media developing copious yellow, butyrous, slimy growth. The macroscopic appearance of *Xanthomonas* cultures on potato dextrose agar or glucose-yeast agar is quite distinctive. Starr and Stephens (1964) have studied the yellow pigment of *Xanthomonas* cultures and named it "Xanthomonas carotenoid" which has not been found in other yellow pigmented bacteria. *Xanthomonas* species readily utilize sugars in synthetic media resulting in acid formation. Production of gas during carbon utilization is absent in authentic *Xanthomonas* species. The inability to utilize salicin is a diagnositic character valuable in identifying *Xanthomonas* species.

The problem of speciation in the genus *Xanthomonas* has become difficult on account of their close similarity in their morphological and biochemical characters. One of the important characters used to identify the species has been the host on which it parasitises and whether it incites leaf-spot infection or systemic vascular disease. It is true that giving undue importance to the host for identifying the pathogen would not be acceptable to many bacteriologists. Dye (1958) on the basis of some experimental studies (which are commented upon later) concluded that the *Xanthomonas* species from one host can be trained and adapted to infect another host. DeLey (1964) without conducting any cross-inoculation studies commented that the so called host-specificity may be a myth. With such strong views expressed by eminent workers, urgent need was felt to carry out some cross-inoculation studies in order to evaluate the importance of host specificity in the taxonomy of *Xanthomonas* species.

In the majority of cases the *Xanthomonas* cultures are usually host-specific and rarely infect more than one host which is closely related taxonomically. For example, *X. corianderi* Srin., Patel and Thirum., on *Coriandrum sativum* L. has been shown to infect in artificial inoculation experiments *Foeniculum vulgare* Gaertn. None of the other umbelliferous hosts tested got infected though the method of inoculation was the same. (Srinivasan, Patel and Thirumalachar, 1961a). *X physalidis* Srin., Patel and Thirum., on *Physalis minima* L. was distinct from *X. vesicatoria* (Doidge) Dowson which also occurs on *Physalis minima* L. (Srinivasan, Patel and Thirumalachar, 1961b). *X. campestris* (Pam.) Dowson and *X. malvacearum* (Sm.) Dowson are known to parasitise more than one host but the host groups are closely related taxonomically. These are

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

thus host-specific in a broader sense and the occurrence of only a few cases of *Xanthomonas* species parasitising more than one host only proves that majority of them is restricted to single host species. This is in contrast to *Pseudomonas solanacearum* Sm. or *P. syringae* van Hall which infect a large number of taxonomically unrelated hosts.

Starr (1959) in his review on bacterial plant pathogens has discussed the problem of speciation in phytopathogenic bacteria and has pointed out the limitations in the use of serology, bacteriophagy, etc., in differentiating species. Dye (1962) has analysed the importance of various determinative tests in the identification of *Xanthomonas* species. Attempts have also been made to carry out a taxonomic analysis of 16 *Xanthomonas* species with the aid of electronic computer (Colwell and Liston, 1961). Studies on the DNA base composition of Xanthomonas also point out that the GC percentage (Guanine+Cytosine) is very uniform over the entire group being in the range of 63-68 per cent Deley, 1964).

One of the important aspects which has been too much commented upon without extensive studies is the inoculation experiments to determine host range. Dye (1958) in carrying out cross-inoculation studies showed that several of the *Xanthomonas* species isolated from different hosts may be made to infect bean plants by training and adapting them. The inoculation was carried out by wounding and spraying with a suspension of the bacterium, and reisolating the bacterium from the necrotic spots after a period of incubation. It has never been stated whether the infection spread from the infection spot following inoculation to other healthy portions of the leaves. As would be pointed out later, establishment of infection would also mean the capacity of the pathogen to multiply in the new site and not merely stay viable at the site where it was introduced into the host by artificial means. This is further illustrated by the work of Logan (1960) who in trying to repeat the work of Dye, also came to the conclusion that *Xanthomonas* are host specific. In his studies on *X. malvacearum* and *X. phaseoli* var. *fuscans* he could not get any conclusive evidence to indicate adaptation of *X. malvacearum* to bean plants after successive inoculations and reisolations, thus contradicting the findings of Dye.

With a view to study host-specificity in *Xanthomonas* species, extensive cross-inoculation studies were undertaken and the results are presented in this paper.

In the first set of the experiments, seedlings of *Argemone mexicana* L. were raised in pots and around it healthy seedlings of *Spermacoce hispida* L., *Lochnera pusilla* Schum., *Blepharis molluginifolia* Pers. and *Tribulus terrestris* L. were planted. Under natural conditions, each one of these host plants is susceptible to infection by a *Xanthomonas* species. A 48-hour old virulent culture of *Xanthomonas argemoneae* Srin. Patel and Thirum. (Srinivasan, Patel and Thirumalachar, 1962a) grown on yeast glucose agar medium was suspended in sterile water to which Tween-80 in 1 : 1000 concentration was added as a spreader, Under optimum conditions of humidity and temperature, the plants were

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

inoculated with an atomiser provided with a powerful air jet. The plants were observed periodically to record the appearance of disease symptoms. It was seen that *A. mexicana* seedlings showed infection of the leaves extending down the hydathodes after 5-7 days and by the 15th day collapsed completely. None of the other plants showed any sign of infection and remained completely free from disease symptoms.

In the next set of experiments, young unfolding leaves of the different hosts were injured by gently rolling the spiny fruits of *Xanthium strumarium* (Compositae) on both the surfaces. By this technique, very fine and delicate punctures were produced on the leaf surface. The inoculation with *X. argemoneae* was carried out as in the previous experiment. Observations at the end of 10 days indicated that besides heavy infection on *A. mexicana*, symptoms were also noticed on some of the delicate plants with thin leaves such as *Lochnera pusilla* and *Spermacoce hispida*. On closer examination it was found that these were wound spots which had dried up simulating in a general way true infection spots. Microscopic examination by sectioning the spot showed darkening of the host cells in the injured region surrounding a cavity. Since the bacterial suspension had been sprayed, bacteria could be recovered from the injured and non-injured portions of the plant in a manner analogous to the recovery of *Xanthomonas* species from soil, plant debris, etc., in studies on the overwintering or oversummering of the pathogen. Some plants of *L. pusilla* and *S. hispida* showing the 'symptoms' were kept under periodic observation for two months during which period the plants were frequently sprayed with jets of sterile water. This was done to facilitate possible infection of young, unfolding leaves and spread of the disease. In carefully planned replicates of the experiment, no fresh infection symptoms developed on the younger leaves and as such there was no further spread of the disease on them and it became clear that a wound type of infection failed to spread further unlike in the case of true pathogenesis.

In the next series of inoculation experiments, a large number of potted seedlings of *Phaseolus vulgaris* L., *Capsicum annuum* L., *Coriandrum-sativum* L. and *Ricinus communis* L. were kept on a terrace away from the plants growing in the field. In the first experiment, the plants were kept in rows and in the subsequent ones kept grouped together in a randomised manner. The plants were sprayed using a powerful jet atomiser with water suspension of a virulent slant culture of *Xanthomonas phaseoli* (EFS) Dowson. The inoculations were carried out during the monsoon months when there is a high degree of humidity in the atmosphere for several days at a stretch. The plants were similarly inoculated with a virulent culture of *X. phaseoli* every alternate day for 20 days so that in all there were 10 inoculations with the bacterial culture. The results of this experiment indicated that the plants of *P. vulgaris* showed infection symptoms within 8 days of commencement of the inoculation experiments whereas the other plants failed to show any symptoms even 25 days after the last inoculation, when the experiment was discontinued. Similar results were obtained with *X. vesicatoria* when the experiment was repeated under identical

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

conditions. In this case only *Capsicum annuum* plants became infected, while the others failed to show any infection symptoms. It was also observed repeatedly that many of the plants like *Coriandrum sativum* and *Physalis minima* having thin, delicate leaves showed yellowing and tip-burning as though affected by sun-scald when sprayed with broth cultures of the organisms. These symptoms caused by either media constituents of the broth or by toxic metabolites produced by the growing bacteria may often give a false impression of successful infection of the host. In this connection mention may be made of the infection on *Pennisetum typhoideum* Rich. named by Rajagopalan and Rangaswami (1958) as *Xanthomonas penniseti*. This organism is a saprophytic bacterium and not a species of *Xanthomonas*. In liquid cultures containing sugar, acid is produced. The infection symptoms reported by Rajagopalan and Rangaswami (1958) in artificial inoculation experiments could only be explained as due to the scalding effect of the acidic broth culture sprayed on the leaves. It is, therefore, very essential that the bacterial suspension should be totally free from the metabolic products or media constituents which may cause undesirable damage to the host tissue. In the inoculation experiments it is desirable to simulate the natural mode of dissemination of the pathogen which takes place by the slow dissolution of the crust-like bacterial slime on an infection spot by wind-blown rain water droplets and its getting splashed from one plant to another. Also, when plants are maintained for prolonged periods under high humidity conditions during artificial inoculation experiments, care must be taken to avoid excessive water-soaking of the leaves since a wet rot may ensue and even saprophytic bacteria entering the substomal space in the damaged tissue may produce symptoms resembling a successful infection.

Based on these experimental results and data on several other inoculation experiments not reported herein, we have come to the following conclusions :

(i) It is necessary to differentiate between a wound spot and a true infection spot. The former type of spot produced by wound inoculation does not produce a disease in the real sense of the term because there is no further spread of infection symptoms. The pathogen is merely harboured inside the necrotic tissue and may live on the dead host cells as a saprophyte and upon reisolation from these spots can be readily brought into culture. This does not mean that the infection cycle is completed and Koch's postulates are fulfilled.

(ii) *Xanthomonas* species are host-specific in the same broad sense of the term as in the case of many other plant and human pathogens. For example, *Madurella mycetomi* causing madura foot in man can be introduced subcutaneously beneath the skin in a rat using a trocar. The fungus lives there and even forms a nodule and it can be recovered even after a period of several months. However, when we speak of pathogenicity of *M. mycetomi*, man is the only host and the fungus is host-specific. No mouse is ever known to be affected naturally by madura foot. Similarly many saprophytic fungi may be inoculated into the peritoneum of a mouse where it causes only a slight foreign body reaction. The fungus can be recovered from the peritoneum after several

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

months but the fungus is non-pathogenic. Hence the mere isolation of an inoculated organism, often causing slight foreign body reaction cannot be considered as proof of pathogenicity.

We definitely know that *Xanthomonas punicae* reported by Hingorani and Singh (1959) on *Punica granatum* L. is not a *Xanthomonas* species. It is associated with *Sphaceloma punicae* which forms necrotic specks. *X. punicae* was isolated during the month of May when the day temperatures are over 110°F and the atmospheric humidity is at its lowest, and when no bacterial diseases can be expected to multiply and spread infection. Dye (1958) has used this organism in his studies and was able to inoculate bean plants through wounding and recovering the bacterium again. This only confirms that such experiments may not prove the pathogenicity of the organism to determine the host range.

(iii) All the differentiating characters for separating the *Xanthomonas* species have not been exhausted as indicated by the studies on the action of antibiotics on different species of *Xanthomonas* (Thirumalachar, *et al.*, 1956 ; Padhya, *et al.*, 1963). The different species showed varying degrees of sensitivity to the antibiotics used. It is, therefore, desirable to look for more distinguishing characters rather than concluding on the basis of our present meagre knowledge.

(iv) In one of the areas where *X. lochnerae* Patel, Thirum and Bhatt on *Lochnera pusilla* Schum. and *X. cleomei* Patel. Abhy. and Thirum., on *Cleome monophylla* L. occurred endemically, the whole area was cleared and earthed up with soil brought from a new place. All the plants were killed and buried one to two feet below the surface. The diseases now have entirely disappeared from the area and the new host plants now growing in the fresh soil have not shown even a single infection spot. It, therefore, appears that if in an endemic area, the host is destroyed, the *Xanthomonas* species would also disappear and no new *Xanthomonas* infection spot would come from another host genus or species. This perhaps explains the success in the eradication programme carried out in the United States for the citrus canker. If any other *Xanthomonas* from hosts such as peach, cabbage, almond, bean or peas could infect *Citrus*, the entire citrus plantations in U. S. A. would have been plastered with citrus canker. The very fact that the disease is still rare or absent shows that the *Xanthomonas* species are host-specific in the sense already explained and host specificity can be made use of in the classification of the species.

(v) Observations made on the epidemiology and natural spread of bacterial diseases also seem to argue against the adaptability of one pathogen to several hosts. For instance, thousands of acres of plants like carrot, poppy, etc. are grown in India and no *Xanthomonas* leaf spots have been observed. If there was adaptation from one host to another, this should have occurred during the hundreds of years the plants have been under cultivation.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Whenever a new disease has been seen, as in the case of the bacterial blight of rice, it has always been possible to trace it to some previous source of infection. If the *Xanthomonas* species are not host specific, and could spread from one host genus to another, there should have been bacterial infection on a very large number of economically important plants, thereby vitiating the need for any Plant Quarantine laws for bacterial diseases incited by *Xanthomonas*. This is not the case, however, if one can remember the case of citrus canker in the United States.

(vi) The taxonomy of the lower groups of organisms can be interpreted in many ways and arguments can always be put forth to justify these interpretations. The main purpose of classification is to aid in differentiating individual forms without sacrificing the basic biological principles. If all the *Xanthomonas* species are lumped together into a few composite species on the basis of our present limited knowledge, then we would have to lift all Quarantine laws, since the diseases on hundreds of hosts would have to be considered as being incited by one species. We, however, know that many of the diseases incited by *Xanthomonas* species are still regional and Quarantine laws must be enforced to prevent spread of the diseases on economic plants. Similarly, by reducing the present species to *forma speciales*, or denoting them by numbers, no new knowledge or advantage is gained, except for some personal satisfaction to the author making these changes.

Baria Science Institute, Navsari
and
Hindustan Antibiotics Ltd.,
Pimpri, near Poona.

References

COLWELL, R. R. AND J. LISTON (1961) Taxonomic analysis with the electronic computer of some *Xanthomonas* and *Pseudomonas* species. *J. Bact.* **82** : 913-919.

DELEY, J. (1964) *Pseudomonas* and related genera. *Ann. Rev. Microbiol.* **18** : 17-46.

DYE, D. W. (1958) Host specificity in Xanthomonas. *Nature, Lond.* **182** : 1813-1814.

——————(1962) The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *N. Z. J. Sci.* **5** : 393-416.

HINGORANI, M. K. AND N. . SINGH (1959) *Xanthomonas punicae* sp. nov. on *Punica granatum*. *Indian J. agric. Sci.*, **29** : 45-48.

LOGAN, C. (1960) Host specificity of two *Xanthomonas* species. *Nature, Lond.*, **188** : 479-480.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PADHYA, A. C., M. K. PATEL, AND M. J. THIRUMALACHAR (1963) In vitro activity of some antibiotics on twelve species of *Xanthomonas* in India. *Hindustan Antibiotics Bulletin* **6** : 6-8.

RAJAGOPALAN, C. K. S. AND G. RANGASWAMI (1958) Bacterial Leaf-spot of *Pennisetum typhoides Curr. Sci.*, **27** : 30-31.

SRINIVASAN, M. C., M. K. PATEL AND M. J. THIRUMALACHAR (1961a). A bacterial blight disease of coriander. *Proc. Indian. Acad. Sci.* B, **53** : 298-301.

————————, M. K. PATEL AND M. J. THIRUMALACHAR (1961b). A new bacterial blight disease of *Argemone mexicana*. *Proc. Nat. Inst. Sci. India*, B **27** : 103-107.

———————————————————————— (1962) Two bacterial leaf-spot diseases of *Physalis minima* and studies on their relationship to *Xanthomonas vesicatoria* (Doidge) Dowson. *Proc. Indian Acad. Sci.*, B, **54** : 93-96.

STARR, M. P. (1959) Bacteria as plant pathogens, *Ann. Rev. Microbiol.*, **13** : 211-238.

———————— AND W. L. STEPHENS (1964) Pigmentation and taxonomy of the genus *Xanthomonas*. *J. Bact.* **87** : 293-302.

THIRUMALACHAR, M. J., M. K. PATEL, N. B. KULKARNI AND G. W. DHANDE (1956) Effects in vitro of some antibiotics on thirty two *Xanthomonas* species occurring in India. *Phytopathology* **46** : 486-488.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TOXICITY, PHYSICAL PROPERTIES AND BIOLOGICAL SPECTRUM OF CERTAIN COPPER AND ORGANIC FUNGICIDES

A. P. MISRA AND T. B. SINGH

(Accepted for publication May 31, 1965)

Abeygunawardene and Peiris (1958) tested the fungistatic efficacy of several fungicides against *Piricularia oryzae* Cav. and found differences in ED 50 values for spore germination between Bordeaux Mixture (5:5:50) and Dithane Z-78. Moreau and Moreau (1961) tested several compounds with similar chemical structure and found that in spite of all these similarities they possessed widely different fungicidal properties.

In the present studies a comparative study was made of the toxicity, physical properties and biological spectrum of three copper fungicides. Bordeaux Mixture (5 : 5 : 50), blitox-50 and Fytolan (copper oxychloride formulations with 50 per cent metallic copper) and two organic fungicides, dithane Z-78 (zinc ethylene bis dithiocarbamate, 65 per cent) and captan (N-trichloro-methyl-thio-tetra hydrophthalamide, 50 per cent), with a view to assess their relative merits.

MATERIALS AND METHODS : *Preparation of spore suspension* : Spores of *Alternaria tenuis* Auct. (which was used as test organism) were taken from 10 day old cultures. Sterilized distilled water was poured in tubes, the surface of the culture scraped by sterilized rubber 'policeman' and by decantation of spore-mycelium-suspension through a double layer of sterilized cheese cloth, a suspension purely of spores was obtained. By dilution with distilled and sterilized water, the concentration of spores in the suspension was adjusted to approximately 50,000 per ml.

Preparation of fungicidal suspensions :

One gram of each fungicide was suspended in 100 ml. of water to get a concentration of 1 per cent (by weight of the fungicide), serial dilutions were made by adding distilled and sterilized water to the stock solution.

Spore germination :

The fungicidal suspensions 0.1 ml. were placed in circles of 1 sq. cm. in area drawn with wax pencil on cellulose nitrate-coated glass slides. Later 0.1 ml. of spore suspension was placed in this area after the drying of the fungicide to maintain its original concentration.

Slides were incubated by keeping them in wooden slide-box lined with wet filter paper at 28°C inside an incubator for 24 hours.

Two hundred spores were counted in each replicate for germinated spores. The germination was defined as the production of a germ tube

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

exceeding its width. A correction for natural mortality was made when it was more than 2 per cent.

The data of spore germination were analysed in the manner described by Bliss (1935). Inhibition of spore germination, expressed as probits, were plotted against log-dose of the fungicide. The relative effectiveness of fungicides was compared by calculating the amount of fungicide required for inhibiting 50 per cent spore germination (ED 50 values) in μg./sq. cm.

Incorporation of fungicides in the medium :

Desired quantities of fungicides were incorporated in molten agar medium in petri dishes in a suspension form. Thorough mixing was obtained by tilting the petri dish from side to side.

Measurement of the radial growth of colony :

In plate cultures the linear (radial) growth of the colony was measured in two directions at right angles to each other and the average was taken as the diameter of the developing colony in mm. Inhibition percentage of growth in the presence of fungicide was determined by comparing with the growth in control.

Estimation of sporulation :

The extent of sporulation in plate cultures were estimated after the method of Peiris (1947) partially modified by Mishra (1955). Mycelial bits 5 mm. in diameter were cut from the medium at 3 different radii and spores suspended in a known quantity of sterile water. The spores were counted with the help of haemacytometer.

Suspensibility :

The suspensibility of fungicides was determined by placing 50 ml. well-stirred suspension representing ED 50 in measuring cylinders and ascertaining the toxicity of the top layer after 15, 30, 60, 120 and 240 minutes by the method described for spore germination. The fungicide having maximum toxicity after maximum times was considered to have maximum suspensibility and *vice versa*.

Coverage :

Eleven drops each of 0.05 ml. suspension, were placed from a distance of 2 mm. on well dried and greese-free glass plate in 40 sq. cm. for each fungicide. Comparision was made on the basis of percentage area covered by each fungicidal drop (suspensions of ED 50 values were used for the test).

Drying :

0.1 ml. suspensions of fungicides capable of producing 50 per cent response to spores of *A. tenuis*, were placed on glass plate and spread to cover an area of 1 sq. cm. for each drop. The time required for drying each drop was noted. Five drops were counted for each fungicide.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Rain resistance :

Three, 0.1 ml. drops of suspensions of fungicides (representing ED 50) were placed on each slide and spread mechanically to cover 1 sq. cm. areas and dried for 12 hours. Eight slides were thus prepared for each fungicide. Of these 5 were kept as control. Remaining 35 slides were placed under shower with a bore size 1.0 mm. at a distance of 2 ft. making angles of approximately 45°. First lot of 5 slides (one from each fungicide) was drawn out after 5 minutes. Second, third, fourth, fifth, sixth and seventh lots were drawn after 10, 20, 30, 40, 50 and 60 minutes respectively. All the slides were dried and assayed for their toxicity to spore germination by the method described earlier. Reduction in ED was taken as the toxicity of fungicides reduced due to rain. Fungicide with minimum reduction in ED showed maximum rain resistance.

Translocability :

Seeds of paddy were soaked in fungicide suspensions of ED 50 values for 24 hours, washed thoroughly, crushed and tested for their toxicity to spores of *A. tenuis*.

One month old paddy seedlings were kept in the above-mentioned suspensions by dipping their roots for 7 days. Extracts from stem and leaves, treated and untreated, were examined for their toxicity. Increased toxicity of seed, stem and leaf was taken as an indication of the presence of the toxicant.

Biological spectrum :

Suspensions capable of producing 50 per cent inhibition of spore germination of *A. tenuis* were tried against spores of *A. solani*, *A. brassicae*, *Helminthosporium oryzae*, *Erysiphe polygoni*, *Peronospora pisi*, urediospores of *Puccinia sorghi* and *Uromyces fabae*. Fluctuation in original ED was considered to be due to varied behaviour of different fungal spores towards the fungicides. Fungicide showing broadest spectrum inhibited maximum germination of maximum fungal spores.

RESULTS : *Effect of fungicides on spore germination of Alternaria tenuis* Auct :

Data on the percentage inhibition of spore germination of *A. tenuis* in different concentrations of fungicides have been illustrated in Fig. 1.

Captan inhibited maximum spore germination, followed closely by dithane Z-78 and Bordeaux mixture. Blitox and fytolan were least efficacious. The positions of the log-probit dose response curves (DR curve) of fungicides (ED 50 values) and their slopes were also calculated. (Table 1).

Captan was effective in inhibiting 50 per cent spore germination at minimum dose of 6.309 ug./sq. cm. as against 131.830 ug./sq. cm. of fytolan which was least effective. The order of efficacy of other fungicides was in the order indicated above.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

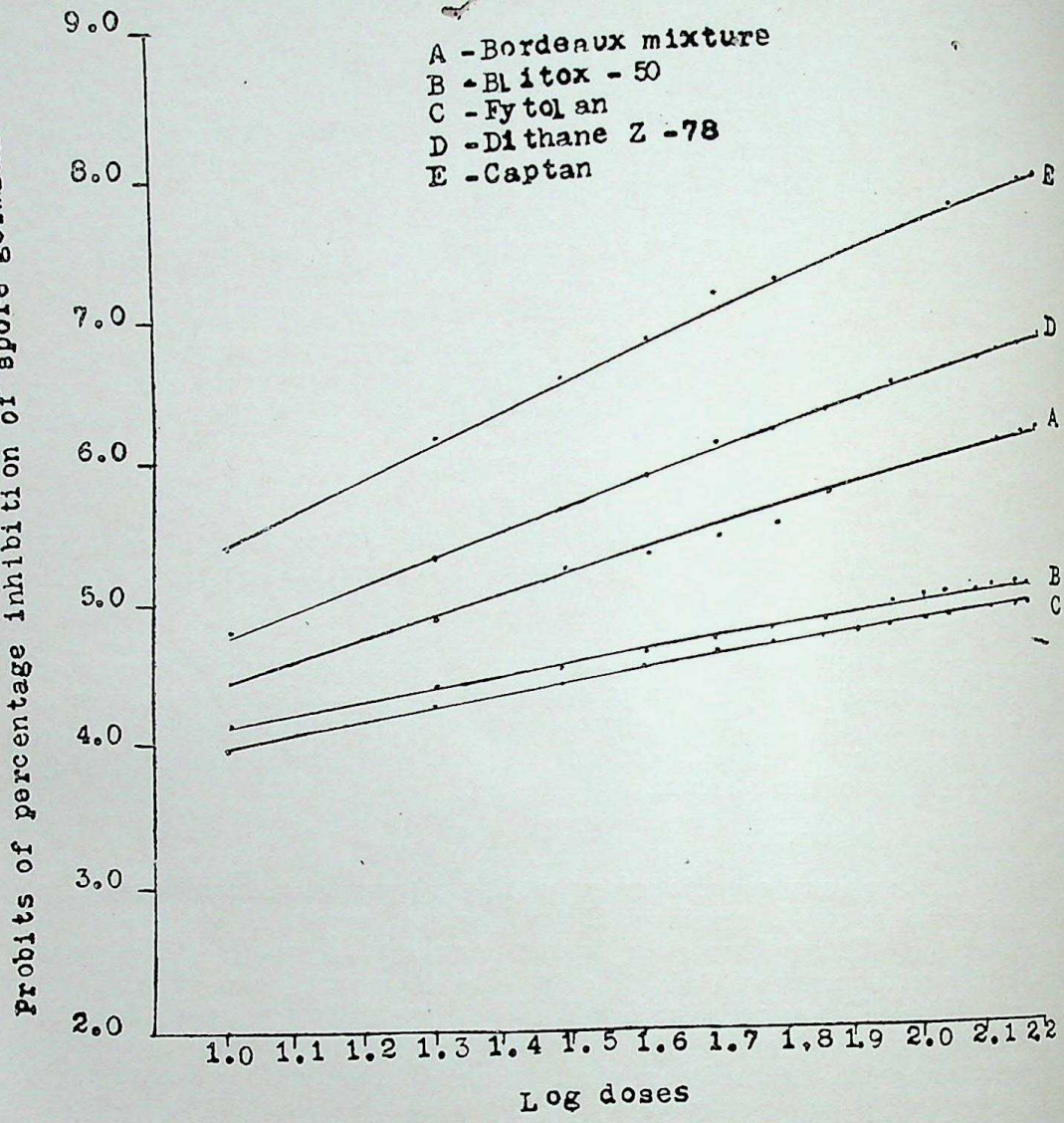


Fig. 1. DR. curves of fungicides using *Alternaria tenuis* spores as test organism

TABLE 1 : Amounts of fungicides in ug./sq. cm. required to inhibit 50 per cent spore germination of *A. tenuis* (positions of DR curves and slopes in degrees.)

Fungicides	ED 50 values	Slopes in degrees
Bordeaux mixture	22.699	17°
Blitox-50	106.666	9°
Fytolan	131.830	10·5°
Dithane Z-78	13.062	19°
Captan	6.309	24°

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

As is apparent from the table, the DR curve for captan was steepest followed in order by dithane, Bordeaux mixture, fytolan and blitox-50.

The effect of fungicides on mycelial growth of *A. tenuis* in potato dextrose agar medium was also investigated. The results were similar to those of spore germination. Maximum inhibition of mycelial growth was observed in captan, followed by dithane Z-78 and Bordeaux mixture, with minimum in blitox and fytolan.

Effect of fungicides on sporulation of A. tenuis :

To determine the effect of fungicides on sporulation of *A. tenuis* the fungus was grown on PDA, in which the different fungicides were incorporated at 500 ppm., 1000 ppm. and 1500 ppm. by weight. The extent of sporulation was estimated with the help of haemacytometer after 100 hours as described under Materials and Methods.

The data are summarized in Table 2.

TABLE 2 : Number of spores of *A. tenuis* in different concentrations of fungicides.

Fungicides	Number of spores per plate in millions at		
	500 ppm.	1000 ppm.	1500 ppm.
Bordeaux mixture	4.0	3.2	1.3
Blitox-50	8.3	8.0	7.0
Fytolan	8.1	7.5	6.5
Dithane Z-78	3.6	3.7	4.3
Captan	2.7	1.4	0.4
Control	6.7		

Among the various fungicides, captan was most efficacious in reducing sporulation, followed closely by Bordeaux mixture, dithane Z-78 and the copper oxychloride formulations.

Suspensibility (Sedimentation) :

Fungicidal suspensions of ED 50 values (capable of producing 50 per cent inhibition of spore germination of *A. tenuis*) were used. After certain time intervals the residual toxicity of the supernatant liquid was determined. The data are summarized in Table 3.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : ED values of fungicides at intervals of 15, 30, 60, 120 and 240 minutes after sedimentation.

Fungicides	Initial ED	ED values after sedimentation at intervals of— 15 min.	30 min.	60 min.	120 min.	240 min	per cent toxicity retained
BM	50	45.16	30.16	22.13	17.83	9.83	19.66
Blitox-50	50	39.16	27.50	20.50	14.66	6.50	13.00
Fytolan	50	40.50	28.00	20.66	14.83	7.00	14.00
Dithane	50	38.00	24.83	18.83	12.50	6.00	12.00
Captan	50	42.00	29.83	21.16	18.80	7.83	15.66

Bordeaux mixture retained maximum toxicity 19.66 per cent and was followed in order by captan 15.66 per cent, fytolan 14.00 per cent blitox 13 per cent and dithane 12 per cent.

Coverage :

Coverage of fungicides was estimated on the basis of area covered by drops of fungicidal suspensions of ED 50 values on glass surface. The results are summarized in Table 4.

TABLE 4 : *Percentage area covered by fungicidal drops.

Fungicides	Total area of each treatment in sq. cm.	Area covered by 11 drops in sq. cm.	Percentage of area covered
Bordeaux mixture	40	11.131	27.829
Blitox	40	9.560	23.901
Fytolan	40	10.620	26.560
Dithane Z-78	40	11.876	29.690
Captan	40	12.806	32.017
Water (Distilled and sterilized)	40	9.929	24.823

*Average of three replicates.

Coverage was maximum in captan, followed by dithane Z-78, Bordeaux mixture, fytolan and blitox-50.

Drying of the fungicides :

Data on the time taken by drops of fungicidal suspension of ED 50 values to dry in shade and sunlight have been presented in Table 5.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 5 : Time taken for drops of fungicidal suspensions to dry in shade and sunlight.

Fungicides	*Time taken by 0.1 ml. drops to dry in minutes	
	In shade	In sunlight
Bordeaux mixture	122.00	57.66
Blitox-50	133.80	67.83
Fytolan	139.00	68.83
Dithane Z-78	102.00	48.66
Captan	101.20	46.16
Water	50.00	42.33

*Average of five replicates.

Captan and dithane were more or less similar and dried earlier than Bordeaux mixture, blitox-50 and fytolan. On the basis of the above data the fungicides were placed in the following order of their efficacy.

Captan > Dithane > Bordeaux mixture > Blitox-50 > Fytolan

Rain resistance :

The effect of artificial rain on residual toxicity of dried deposits of fungicides was determined according to the method described under Materials and Methods. The data are presented in Table 6.

TABLE 6 : Effect of artificial rain on residual toxicity of dried deposits of fungicides.

Duration of rain in minutes	*Percent inhibition of spore germination of *A. tenuis*.				
	Bordeaux mixture	Blitox-50	Fytolan	Dithane	Captan
5	20.33	10.00	9.66	11.66	13.00
10	15.33	7.00	6.66	8.66	11.00
20	14.33	6.00	5.66	6.33	7.33
30	13.36	3.66	3.33	6.00	7.00
40	12.00	2.66	2.33	3.00	5.66
50	11.00	2.00	1.33	2.66	4.33
60	10.66	0.66	0.33	1.66	2.66
Control (no water)	50.00	50.00	50.00	50.00	50.00

*Average of three replicates.

Bordeaux mixture resisted the washing-out effect most. Though it lost 70 per cent of its initial toxicity after 10 minutes of rain, yet it

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

retained 10.66 per cent of toxicity after 60 minutes. It was followed by captan, dithane, blitox and fytolan.

On the basis of their property of rain resistance, the fungicides were placed in the following order of their efficacy.

Bordeaux mixture > Captan > Dithane > Blitox > Fytolan

Translocability of fungicides :

An experiment was made to find out whether the fungicides were translocated in plant parts according to the method described under Materials and Methods. The data are presented in Table 7.

TABLE 7 : Effect of extracts obtained from treated seeds and seedlings on the germination of spores of *A. tenuis*.

Fungicides	Per cent inhibition of spore germination in		
	Extracts from seed soaked for 24 hrs. in fungicide	Extracts from stem of seedlings whose roots were dipped for 7 days in fungicide	Extracts from leaves of seedlings whose roots were dipped for 7 days in fungicide
Bordeaux mixture	3.33	10.66	4.66
Blitox	3.00	11.00	5.00
Fytolan	3.00	11.33	5.33
Dithane Z-78	9.00	22.33	7.00
Captan	26.33	40.00	10.00
Control	3.00	11.33	5.33

None of the copper fungicides were translocated either through the seeds or seedling tissues. The other two fungicides, viz., captan and dithane Z-78 were, however, translocated in all the three types of plant tissues, seed, stem, and leaf.

Biological spectrum :

To determine the biological spectra of fungicides an investigation was carried out according to the method described under Materials and Methods. The data have been summarized in Table 8.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 8 : ED values of fungicidal suspensions against different fungi.

Fungi	Fungicides				
	Bordeaux mixture	Blitox-50	Fytolan	Dithane Z-78	Captan
A. tenuis	50	50	50	50	50
A. brassicae	48.50	47.80	47.60	49.00	49.50
A. solani	47.00	46.67	46.00	48.26	48.85
H. oryzae	44.52	42.22	42.00	47.65	47.95
P. sorghi	18.43	8.44	8.00	45.25	46.00
U. fabae	20.60	9.30	8.74	46.72	47.52
E. polygoni	9.04	0.00	0.00	59.63	59.69
P. pisi	46.16	44.90	41.00	50.65	51.90

It is apparent that captan and dithane were most effective against all the fungi tested. The copper fungicides were effective against *Peronospora pisi*, *Helminthosporium oryzae*, *Alternaria solani* and *Altarnaria brassicae* but the results were different in case of powdery-mildew and the rust fungi.

SUMMARY

Relative studies on the toxicity and physical properties of three copper and two organic fungicides were made. Their ED 50 values, using *Alternaria tenuis* Auct. as test organism were as follows : Bordeaux mixture 22.699 μg./sq. cm., blitox-50, 106.666 μg./sq. cm., fytolan, 131.830 μg./sq. cm., dithane Z-78, 13.062 μg./sq. cm. and captan 6.309 μg./sq. cm.

Captan inhibited 50 per cent mycelial growth of *A. tenuis* in culture medium at the rate of 15,996 μg./sq. cm. of the medium, whereas dithane, Bordeaux mixture, blitox, and fytolan required 33.111, 36.670, 95.940 and 120.230 ug./sq. cm. respectively.

The rate of sedimentation was minimum in case of Bordeaux mixture, followed by captan, fytolan, blitox and dithane Z-78. Bordeaux mixture retained 19.66 per cent toxicity after 240 minutes as against Dithane Z-78, which retained 12.00 per cent.

Captan had the maximum spreading capacity followed by dithane, Bordeaux mixture, blitox and fytolan.

Captan required minimum time for drying both in shade and sunlight (101.20 and 46.16 minutes), dithane required slightly more time followed by Bordeaux mixture, whereas blitox and fytolan dried

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

after 133.80 and 139 minutes in shade and 67.83 and 68.83 minutes in sunlight respectively.

Rain resistance was maximum in Bordeaux mixture, followed by captan, dithane, blitox and fytolan. Bordeaux mixture resisted the washing-out effect of rain to the maximum and retained 10.66 per cent of original toxicity even after 60 minutes of rain.

ACKNOWLEDGEMENT : Thanks are due to Sri R. S. Roy, Principal, Bihar Agricultural College, Sabour for providing the necessary facilities for this work.

Department of Mycology and Plant Pathology,
Bihar Agricultural College,
Sabour (Bhagalpur)

REFERENCES

ABEYGUNAWARDENE, D. K. W. AND J. W. L. PEIRIS, (1958) Studies on the fungicidal control of rice blast. I. The fungistatic efficacy of certain organic and inorganic fungicides on *Piricularia oryzae*. *Indian Phytopath.* **11** : 116.

BLISS, C. I. (1934) The method of probits. *Science.* **79** : 38.

MISHRA, J. N. (1955) Morphological, physiological and pathological studies of *Botrytis cinerea* Pers. *Proc. Bihar Acad. Agri. Sci.* **4** : 19-32.

*MOREAU, C. AND M. MOREAU, (1961) Evaluation of the comparative fungicidal activity of substances with similar chemical structure. *Bull. Soc. Myc. Tr.* **77** : 273-287.

*PEIRIS, J. W. L. (1947) *The Botrytis disease of gladiolus together with a physical study of certain* Botrytis spp. Thesis for Ph. D., Univ. of London.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON SUGARCANE RUST IN INDIA*

M. L. SAHNI AND B. L. CHONA

(Accepted for publication April 24, 1965)

INTRODUCTION : Till recently, sugarcane rust had been considered of rare occurrence and regarded as a disease of no economic importance. During recent years, however, it has become increasingly common and is reported to be causing damage to the crop, particularly to some of the improved cane varieties. In 1950, a serious outbreak of this disease occurred in the State of Bombay, affecting a very popular cane variety, Co. 475 (Chona *et al.*, 1950; Patel *et al.*, 1950). It has, since then, spread to several other varieties in the Peninsular India. In the North, it assumed an epiphytotic form during 1955-56, affecting the September-October planted crop of the variety Co. S. 510 in Jamnabad Farm, Golagokarannath (District Lakhimpur Kheri, U. P.) near the foot hills of Himalayas (Vasudeva, 1956). The affected crop, however, recovered remarkably well by the following October (Chona, 1958).

Though sugarcane rust had been known as far back as 1918, and the specimens were deposited in the Herb. Crypt. Ind. Orient. Indian Agricultural Research Institute, New Delhi (Butler, 1918), no attempts were made to investigate its mode of perpetuation and life-history obviously because the disease was of minor economic importance and of limited occurrence.

In view of its increasing economic importance as a result of its virulence on certain promising cane-varieties, and its serious nature, it was considered desirable to investigate the disease and to carry out comparative study of the rusts occuring on different *Saccharum* spp. The results are reported in this paper.

MATERIAL AND METHODS : Sugarcane and *Erianthus* rust cultures were obtained from Plant Pathological Substation, Flowerdale, Simla, in the last week of November, 1956. Rust samples on Co. S. 510 cane variety were received from Kichha (Golagokarannath) and Lakhimpur Kheri, (U. P), and on *Saccharum munja* from Kichha in the middle of February, 1957. In addition, the rusts on *S. spontaneum* and *S. arundinaceum* were collected from the fields of the Indian Agricultural Research Institute, New Delhi and Okhla (Delhi), respectively.

The rust cultures were maintained on young plants of variety Co. S. 510 growing in 10-inch pots, as well as on their respective host varieties.

*This paper is based on thesis submitted by the senior author for Associateship Indian Agricultural Research Institute in Mycology and Plant Pathology, New Delhi in 1957.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Before inoculations the plants were thoroughly sprayed with water. The inoculum was applied to both the surfaces of a leaf with a lancet needle. The plants were then put under a humid chamber and were sprayed with water 3-4 times a day. After 48 hours, they were kept outside in the shade and were covered with belljars to give a humid environment and enough light.

A few of the inoculated plants were also maintained in a cool room where the temperature was maintained at about 25°C during the summer months.

During the hot summer months the cultures were maintained outside in a shady place in a humid chamber made of wooden frame 7′ × 5′ covered with gunny-bags on all sides which was kept moist by spraying water on it frequently. Similar type of large gunny-bag humid chambers were used for varietal resistance tests.

Also, the cut-leaf technique, similar to that employed by earlier workers like Arthur (1906, 1908), Waters (1928), Yarwood (1946) etc. was used for maintaining the cultures as well as for testing the varietal resistance and also for collateral hosts study during summer months when it was not possible to lower the temperature of glasshouses, For the cut-leaf method healthy, green leaves were cut into pieces, 2″ to 2½″ long, washed, wiped dry and dipped in rectified spirit (95 per cent alcohol) for a few seconds and then rinsed in several changes of sterile distilled water, before they were placed on sterile petri-plates containing a thin layer of moist cotton wool. One end of the leaf was placed on a glass rod while the other end was allowed to touch the moist cotton pad. The leaves were then finally inoculated with a lancet needle and sprayed with water. The inoculated petri-plates were kept at 24—30°C tamperature range. All the operations, were made under aseptic conditions. Water (with 0.1 per cent "tween") was frequently added to these chambers to maintain high humidity. In addition, the sucrose solution in the petri-dishes was changed every third day.

For morphological studies dry spores were placed on a clean glass slide and a coverslip was placed over it. A few drops of water were then introduced from one side of the coverslip before taking the measurements.

Spore germination studies were carried out on 1 per cent agar and also in hanging-drops in Van Tiegham cells placed at different temperatures. For teleutospores germination hanging-drop cultures or cavity slides were used.

For the histopathological studies, the infected leaf parts were fixed in F. A. A. before processing them for microtome sectioning. The sections were cut 5—10μ thick, stained with Iron alum haematoxylin, counterstained with fast green 1 per cent in 90 per cent ethyl alcohol. Some sections were also stained in safranin 1 per cent in 70 per cent ethyl alcohol, and were finally mounted in canada balsam.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL : A. *Field Observations in Neighbourhood of Delhi* : During the year 1956-57, a number of places in and around Delhi were visited from time to time to record the appearance and presence of the rust on some allied species of *Saccharum* in the field under natural conditions. The leaves of some of the *Saccharum* spp. were found heavily infected by rust during the cooler months. The leaf lamina as well as leaf-sheath were found infected. Rust on *S. spontaneum* was collected from October to May at the Indian Agricultural Research Institute, New Delhi. The affected plants were characterised by orange brown coloured raised pustules, which were amphigenous, linear and elongated. After the rupture of the epidermis, spore masses are exposed and can be seen on the surface as raised sori occurring in elongated mass of spores. The young tender portion of the stem was also, at times, found infected with rust.

Rust on *S. arundinaceum* was collected from November to April, on plants growing along the side of Jamuna Canal at Okhla. The pustules were observed on lamina and leaf-sheath in the form of small, yellow sori. Only the uredostage was noticed on both these hosts (*S. spontaneum* and *S. arundinaceum*).

B. *Morphology—Uredo Stage* : (i) On *Sugarcane* (Co. S. 510) : Dark brown pustules which appear in groups, as linear, minute lesions of light or dark brown colour are visible on both sides of the affected leaf blade and leaf-sheath. The uredospores are borne on long, hyaline, stalks which get detached soon (Fig. 1). These are cinnamon-brown coloured, variable in shape, all gradations occurring in between ovate to pear-shaped forms. The wall is hyaline, finely echinulate with 4-5 germ pores usually located at the equatorial region and encloses reddish brown contents. Paraphyses, which are hyaline to golden in colour and club-shaped or capitate, are usually in abundance at the margins of uredosori or intermixed with the spores.

(ii) On *Erianthus fulvus* Nees : Uredosori are amphigenous, linear, upto 5 mm. in length, 0.5 mm. broad, amber coloured. Uredospores are ovoid to pyriform, with thin epispore, finely echinulate, with 4-5 large distinct pores either equatorial or scattered, and ochreous coloured. Paraphyses are capitate, thickened apically in the head, hyaline, golden or purplish-brown and numerous.

(iii) On *Saccharum spontaneum* L. : Uredosori are amphigenous or hypophyllous, elongated or linear, 0.5 to 1.0 mm. long, and brown coloured ; uredospores are variable in shape usually oblong to sub-globose (Fig. 2). The epispore wall is uniform or often thickened at the apex, moderately echinulate, with 4 or 5 equatorial germ pores. Paraphyses are inconspicuous, peripheral, cylindric, capitate, hyaline and of golden or brown colour.

(iv) On *Saccharum munja* and *S. arundinaceum* Retz. : Morphologically both these rusts appear to be similar. Uredosori are amphigenous or hypophyllous, orange to yellow in colour. Linear or elongated, 0.5 mm. to 2 mm. long ; uredospores are golden to orange-yellow in colour,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

variable in shape, oval to pyriform (Fig. 3) with wall uniformly thickened echniulate, with 4-5 equatorial germ pores. Paraphyses are peripheral, capitate, hyaline and golden in colour. The measurements of the uredospores obtained from different hosts and localities are given in Table 1.

TABLE 1 : Measurement of uredospores obtained from different hosts and localities

Host	Locality	Average size and range of uredospores collected from different hosts (Average of 50 spores) (μ).
Erianthus fulvus	Simla	29.0 × 20.0 (22.4 – 38.4 × 16 – 23.9)
Saccharum munja	Khurpia Farm, Kiccha, U. P.	27.5 × 21.2 (16.4 – 41 × 12.3 – 26.6)
S. arundinaceum	Okhla, Delhi	30.2 × 21.9 (38 – 22.4 × 16.0 – 25.6)
S. spontaneum	IARI., New Delhi.	31.6 × 19.6 (45 – 20.5 × 24.6 – 13.3)
Sugarcane (Co. S. 510)	Pipra Farm, Lakhimpur Kheri, U. P.	31.6 × 22 (38.4 – 22.4 × 25.6 – 17.6)

C. *Effect of Temperature on Germination of Uredospores* : The germination of uredospores was studied at 7 different temperatures viz. 10°, 15°, 18°, 20°, 25°, 30° and 40°C. The final observations, were recorded at the end of 24 hours. In all the cases (Sugarcane (Fig. 1), *Erianthus fulvus* and *S. spontaneum* (Fig. 2), *S. arundinaceum* (Fig. 3), *S. munja*) uredospores germinated through germ pores giving out one or two germ tubes. In the case of sugarcane and *Erianthus* rusts, the uredospores started germinating within 6 to 12 hours while in others the germination started within 6 hours. The germination percentage at different temperatures for the different rusts is presented in Table 2.

TABLE 2 : Germination of the uredospores at different temperatures

Temperature °C	Germination percentage (Average of 3 tests) of the uredospores				
	Sugarcane	*Erianthus fulvus*	*S. spontaneum*	*S. arundinaceum*	*S. munja*
10	3-4	Nil	3-5	2-3	2-3
15	28-33	5-7	35-40	34-39	20-23
18	41-47	12-15	70-76	61-66	56-60
20	71-78	27-30	82-85	80-83	74-80
25	37-40	10-20	60-65	57-62	44-48
30	15-20	4-5	14-22	20-25	20-23
40	Nil	Nil	Nil	Nil	Nil

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 1. *Sugarcane* rust—germinating uredospores.

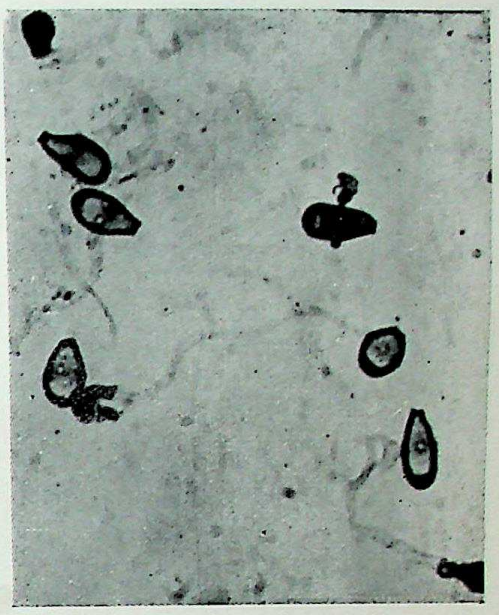


Fig. 2. *S. spontaneum* rust—germinating uredospores.

Fig. 3. *S. arundinaceum* rust—germinating uredospores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

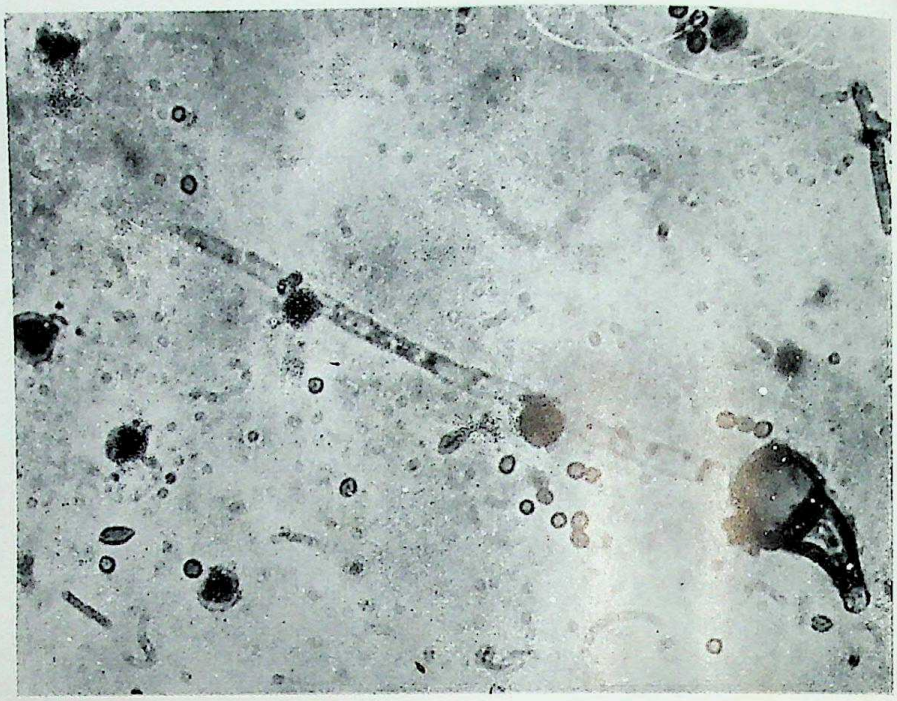


Fig. 5. *Sugarcane* rust—germinating teleutospores.

Fig. 6. *Erianthus fulvus* rust—germinating teleutospores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It would be seen that germination in almost all the cases, started at 10°C and continued upto 30°C. The maximum germination was obtained at 20°C in all the cases, while there was no germination at 40°C. Only in *S. spontaneum* a slight swelling of the apex of germ tube was observed (Fig. 2).

D. *Longevity of Uredospores under Storage* : The rust on sugarcane (Co. S. 510) was obtained from Golagokarannth, (U. P.) on 12th February 1957 and was air-dried overnight at room temperature (18°C). It was then placed in a butter-paper envelope and stored at 8-10°C as well as at room temperature (18—28°C) through February to April. The viability of the uredospores at the time of storage was found to be 69 per cent and it was checked every week by germination tests conducted at 20°C. The results are presented in Table 3.

TABLE 3 : Longevity of uredospores under storage at different temperatures.

Storage period (weeks)	Germination percentage of uredospores stored at	
	8-10°C	18-28°C
0	69	69
1	65	60
2	62.5	52
3	56.1	38
4	45.5	25
5	38	11
6	35	Nil
7	31.5	—
8	18	—
9	12	—
10	5	—
11	2-3	—
12	Traces	—
13	Nil	—

It would be observed from Table 3 that there was a gradual decline in the viability of the spores at both the temperature conditions of storage with the advancement of storage period. The uredospores retained their viability for 12 weeks at 8—10°C and only for five weeks at room temperature (18—28°C).

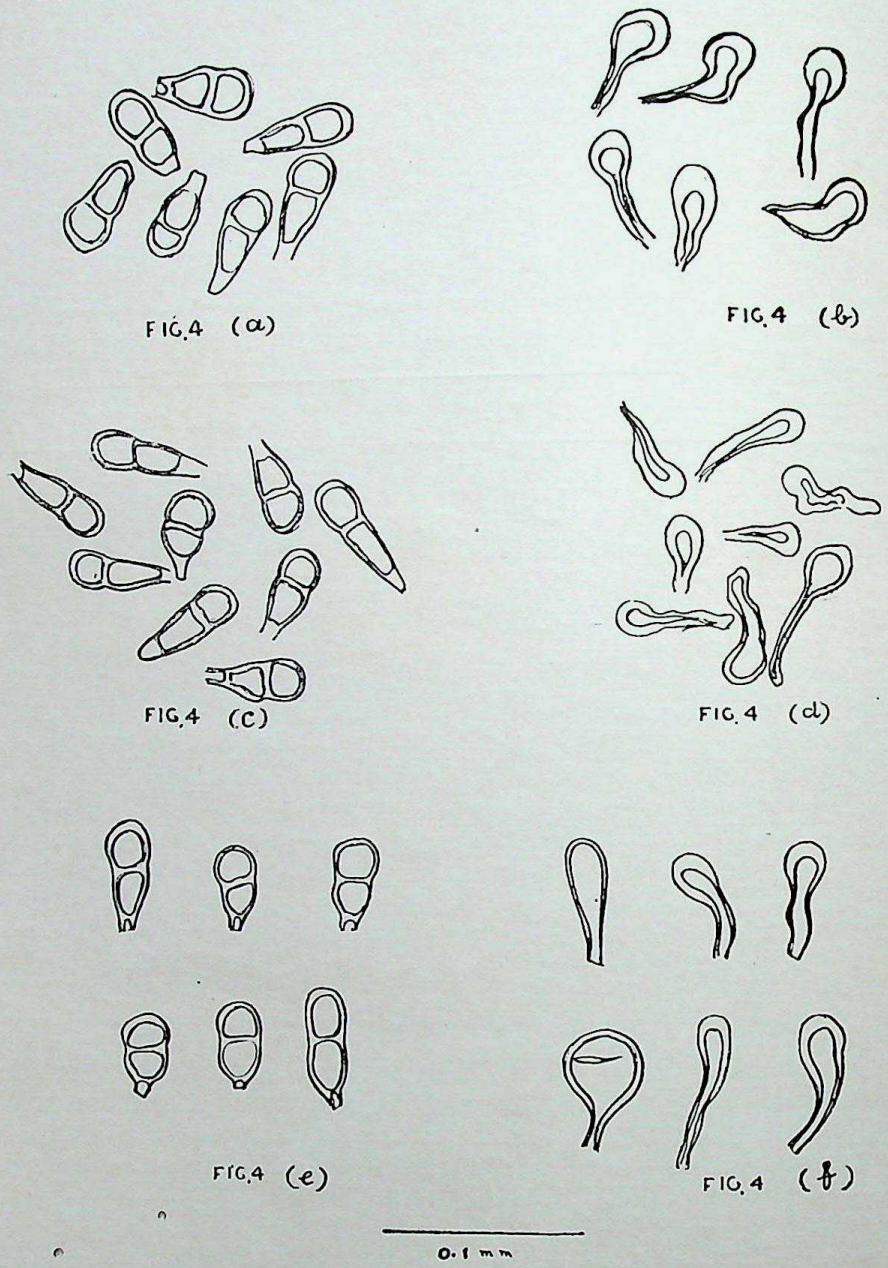
E. *Teleutostage* : *Sugarcane Rust* : The teleutosori are amphigenous, often in groups. The teleutospores (Fig. 4a and e) are bicelled, clavate or club shaped, rounded, or somewhat flattened above and tapering towards the stalk, with the lower cell bulging and longer than the upper cell and with a slight constriction at the septum. Epispore is smooth,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

somewhat thickened at the apex. These are chestnut brown in colour. The pedicel is short, hyaline, brown in colour and persistent. Paraphyses (Figs. 4b and 4f) are similar to those of uredosori.

FIG. 4



Figs. 4a—4f. 4a and 4b. Teleutospores and paraphyses of sugarcane (var. Cos. 510) rust received from Golagokarannath (U. P.). 4c and 4d. Teleutospores and paraphyses of *Erianthus fulvs* rust received from Simla. 4e and 4f. Teleutospores and paraphyses of sugarcane rust received from Simla.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Erianthus fulvus Rust : The teleutosori of the rust obtained from Simla on *Erianthus fulvus* (Fig. 4e and f) are amphigenous or hypophyllous, minute, linear, 5 mm. long and 0.5 mm. broad, subepidermal and dark brown in colour. Teleutospores (Fig. 4c) are bicelled, clavate, round at the apex, narrow, elongated basally, lower cell often double the length of the upper one, slightly contricted at the septum. Epispore thin. hyaline, slightly thickened at the apex; pedical concolorous with the base of spore, moderately thin-walled but usually not collapsing laterally, short, persistent amber coloured. Paraphyses (Fig. 4d) are similar to those of the uredosori. The details of measurements of spores and paraphyses are given in Table 4.

TABLE 4 : Measurements of teleutospores of rusts collected from different hosts and localities

Host	Locality	Average size of teleutospores (μ) and Range (average of 50 spores)					Paraphyses	
		Spores	Upper cell	Lower cell	Stalk	Thickness at apex	Size	Thickness at apex
Erianthus fulvus	Simla	30.1× 17.3 (33— 44.8× 14.4— 19.2)	16.3× 17.3 (14.4— 19.2× 14.4— 19.2)	21.1× 14.4 (16— 25.6× 12.8— 16)	7.0 4.8— 9.6	3.2— 4.6	49.6× 12.5 (32— 67.2× 9.6— 16)	4.5 (3.2— 6.4)
Sugarcane CoS.510	Pipra Farm, Lakhimpur Kheri, U. P.	39.6× 19.2 (51.2— 25.6× 25.6— 12.8)	18.9× 19.2 (25.6— 12.8× 25.6— 12.8)	20.4× 16.9 (28.8— 12.8× 25.6— 12.8)	6.08 (4.8— 9.6)	3.5 (3.2— 4.8)	—	—
Sugarcane CoS.510	Simla	35.2× 18.4 (48.0— 25.6× 24.0— 12.8)	16.7× 18.4 (22.4— 6.4× 24.0— 12.0)	17.4× 16.6 (28.8— 9.6 25.6— 12.0)	5.9× 5.9 (9.6— 3.2× 8.0— 3.2)	—	48.9× 17.6 (73 6— 22.4× 25.6— 9.6)	—

F. *Germination of Teleutospores* : The teleutospore material of sugarcane rust was obtained during the last week of November, 1956, from the Simla Sub-station, where the rust cultures were being maintained during the summer months and that of *Erianthus* rust was collected from naturally infected *E. fulvus* at Simla. These samples were air-dried and kept in envelopes which were stored at 8—10°C. Teleutospores were scraped from dry leaves and after soaking them in water for

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

different periods, they were subjected to different treatments and their germination tested at different temperatures.

Since the teleutospores did not germinate by the usual methods in the months of November or December it was considered necessary to treat them with some chemicals and also to investigate whether the spores were undergoing a period of dormancy. So different experiments were set up on the following lines.

1. Testing of viability of material from time to time in distilled water.

2. Testing of viability of spores in different chemicals to break the dormancy. These experiments are discussed in details below :

It was observed that the spores which were stored at 8–10°C did not germinate upto the end of January but the same material showed some germination in subsequent months (February-March).

This is the first record of germination of teleutospores of sugarcane (Fig. 5) as well as *E. fulvus* (Fig. 6) rust. It would be seen that the teleutospores have a period of dermancy before germination.

G. *Effect of Temperature on Germination of Teleutospores* : The effect of temperature was studied by incubating the teleutospores in drops of distilled water at different temperatures ranging from 10 to 40°C. The observations were recorded every 24 hours and the data are presented in Table 5.

TABLE 5 : Effect of temperature on teleutospore germination.

Temperature °C	Germination (per cent)	
	Erianthus fulvus Rust	Sugarcane Rust
10	Nil	Nil
15	Nil	Nil
18	3	5
20	8-10	7-10
25	Trace*	Trace*
40	Nil	Nil

*Trace = Less than 1 per cent.

It would be seen from Table 5 that teleutospores germinate best at 18–20°C although in every test the germination was scanty. The

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

germination was found to start within seven days in both the rusts. No germination was observed till the fifth day. The length of the promycelium on the 7th day, in case of *E. fulvus* rust ranged from 95 to 286μ, whereas, for sugarcane rust it was 128 – 275μ. In a few cases, fairly long, sterigmata-like structures were observed at the tips of promycelia. Promycelia were observed to be septate, having four cells, and in some cases bearing four sporidia.

Since the germination of teleutospore was very poor, it was suspected that the spores might be in dormant conditions. An effort was, therefore, made to break the dormancy with chemicals as ammonium chloride 1 per cent, Citric acid 1 per cent, nitric acid 0.01 per cent, hydrochloric acid 0.01 per cent, sulphuric acid 0.01 per cent, potassium hydroxide 1 per cent and oxalic acid 1 per cent. None of the chemicals tested, however, affect the teleutospore dormancy. More elaborate studies are required in this direction in order to obtain better germination of teleutospore.

H. *Inoculation with Teleutospores* : Sugarcane and *Erianthus fulvus* plants were inoculated with germinating teleutospores of sugarcane and *Erianthus* rusts, by picking up single spores with the help of a fine needle under binocular microscope and placing them on the leaf lamina of young plants grown in 10-inch pots as well as on detached leaves of sugarcane and *Erianthus*. But in no case any successful infection was obtained, indicating thereby that the rust is not autoecious

I. *Varietal Resistance Test* : A large number of cane varieties have been reported to be infected with rust. It was, therefore, felt necessary to study the response of other improved varieties to this rust. It was with this aim in view that preliminary rust resistance tests were carried out in the month of April with 22 cane varieties, namely, B. 0.3, 10, 11, 14, 21, 22, 24, Cok. 32, Cos. 186, 443, 510, Co. 449, 513, 514, 515, 517, 527, 622, 851, 859 and 976 available at that time. The inoculations were made on plants grown in 10″ pots as well as on excised leaves. Two varieties, viz. Cos. 186 and Co. 513, in addition to the Co. S. 510, the susceptible control, got infected. The incubation period was found to be 15 and 13 days in case of pot plants and cut leaf respectively.

J. *Collateral Hosts* : Considering the possibility of some wild grasses acting as collateral hosts for the sugarcane rust fungus, a few grasses that were available were tested. These were, *S. spontaneum* *S. arundinaceum* and *Sclerostachya fusca*. Cross inoculation tests were, therefore, conducted with uredospores of sugarcane rust as well as those of rusts on *E. fulvus*, *S. spontaneum* and *S. munja*.

The inoculations were made on leaves of young plants in 10 inch-pots as well as on detached leaves. The results of inoculations with uredospore of *Saccharum* spp. on different grasses are given in Table 6.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 6 : Results of cross-inoculation tests with rusts of sugarcane and other Saccharum spp. on different grasses.

Original host of Rust inoculum	Test-Plant				Incubation period (days)
	S. officinarum Co.S.510	*S. spontaneum*	*S. arundinaceum*	*S. fusca*	
S. officinarum CoS.510	+	—	—	—	10-11
E. fulvus	+	—	—	—	11
S. spontaneum	+	+	—	—	8-10
S. munja	—	—	—	—	---
S. arundinaceum	--	—	+	—	9

(+) Indicates the presence of rust infection.

(—) Indicates the absence of rust infection.

Data presented in Table 6 show that rusts of some other grasses too can infect sugarcane. Among those tested, *S. spontaneum* rust could be easily transferred to sugarcane

Erianthus fulvus rust could also be easily transferred to sugarcane by pot plants inoculation as well as by cut leaf technique. There was, however, difference in the intensity of the rust infection. The infection was rather mild when the inoculum from *E. fulvus* was used compared to that from *S. spontaneum.* It may be due to the viability percentage of the spore as already noted in germination test. On the other hand, the rust of *S. arundinaceum* could neither be transferred to sugarcane nor sugarcane rust to *S arundinaceum.* Due to non-availability of plants of *E. fulvus* and *E. munja* the pathogenicity of sugarcane rust organism on these hosts could not be tested.

DISCUSSION : Useful data has been collected as a result of comparative study of rusts on grasses closely related to sugarcane. Average measurments of uredospores of sugarcane rust obtained from Simla, Golagokarannath (U. P.) and Kichha Farm (U. P.) vary from 29.6 to 31.5 $\mu \times$ 20.3 to 22.4 μ. Uredospores of rust on *Erianthus fulvus* measured 29.0 to 20.0 μ and that on *S. arundinaceum* 30.2$\times$21.9 μ. It would be seen that the measurements almost overlap those of sugarcane rust. Uredospores of *S. spontaneum* rust were found to be 31.6 to 33.2$\times$ 19.6 to 22.2 μ and appeared to be slightly more oblong while those of *S. munja* measured 27.5$\times$21.0 μ and were slightly more spherical as compared to those of sugarcane rust.

Besides the measurements, there are apparently very few morphological differences among these results, except *S. spontaneum* rust in which uredospores have a thickened apex. Uredospores of sugarcane rust and *E. fulvus* rust are almost indistinguishable from each other. The

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

uredospores of these two rusts differ from those of *S. munja* and *S. arundinaceum* rusts only in their colour, since they are reddish brown while the last two are golden yellow. The teleutospores which were found only on *E. fulvus* and sugarcane appear to be almost alike, with spore measurements as 38.1 × 17.3 μ and 39.6 × 19.3 μ, respectively. The first and only record of this stage on *S. spontaneum* was by Butler (1914) from Burma. Indeed, the causal organism of sugarcane rust is still under controversy. However, in the light of present results reported in this paper it appears that the sugarcane rust organism has more affinity with *E. fulvus* rust than *S. spontaneum* rust. Hence, the sugarcane rust with teleutospore can be ascribed as *Puccinia erianthi* Padwick and Khan in conformity with Cummin's view (1953) on its identity. Only more extensive studies would reveal whether all these rusts are varieties of one species or even physiologic forms of one or more varieties.

Teleutostage is the only stage of sugarcane rust which has been described by Chona *et al.* (1950) ; Patel *et al.* (1950) and Vasudeva (1956) from India and various workers from abroad, while *E. fulvus* rust was described by Padwick and Khan (1944). The present morphological studies confirm the findings of Cummins (1953). Germination of teleutospores of sugarcane and *E. fulvus* rust was observed for the first time during the course of present studies. The germination, after dormancy, was observed at 20°C after an incubation of seven days. The germination at 15°C and 25°C was, however, very low.

The teleutospores of sugarcane and *E. fulvus* rust when transferred to sugarcane and *E. fulvus* respectively, failed to infect the two hosts. The experiment wast repeated several times but without any success, indicating thereby that the rust is not autoecious and an alternate host should be looked for. Till an alternate host is discovered the knowledge of the life-history of this pathogen would remain incomplete.

An attempt was made to maintain the rust cultures in a viable form during the summer months at Delhi under a shady place and in high humidity were not successful.

As in other rust, the only feasible method of control at present appears, to be cultivation of resistant varieties, namely, Co.421, 467, 475, 603, 658, 678, 732, 826, 928, Co.S.510 and P.o.J.2878 have been reported to be susceptible to rust. Preliminary tests of varietal resistant were, therefore, undertaken with the sugarcane rust and it was found that out of twenty-two varieties tested only two varieties, namely Co.513 and Co. 186, and the susceptible Control Co.S.510 got infected. Similar trials on a large number of varieties with different isolates can provide a clear picture of varietal response to sugarcane rust.

In collateral hosts study, detached leaf technique was also employed for carrying out cross inoculations. It was observed for the first time that rusts on *S. spontaneum* and *E. fulvus* could easily infect sugarcane. As these grasses are perennial hosts and also occurred in hilly areas, the rust would survive on these hosts in the hills or in shady places at the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

foot hills of Himalayas and may serve as a colletral host to perpetuate the sugarcane rust. More extensive surveys and studies are required to elucidate this aspect further.

SUMMARY

Since about 1950, sugarcane rust which is gradually increasing in India has been studied carefully. In cultural studies the incubation period was found to be 10-11 days both in sugarcane rust and *Erianthus fulvus* rust. Comparative morphological study of rusts on sugarcane and grasses closely related to sugarcane have shown that the causal organism of sugarcane rust is considered as *P. erianthi*.

The uredospores of rusts on sugarcane, *E. fulvus*, *Saccharum arundinaceum* and *S. munja* and *S. spontaneum* germinated freely in water at 10°C to 30°C, the optimum being about 20°C, and the time taken to germinate is within 6 to 12 hours in the first two while uredospores of *S. spontaneum*, *S. munja* and *S. arundinaceum* rust started to germinate within six hours. The uredospores of sugarcane rust, were found to remain viable for 12 weeks at 8-10°C and for 5 weeks at room temperature (18-28°C).

The sugarcane could be infected with uredospores of *S. spontaneum* rust as well as *Erianthus fulvus* rust. Optimum temperature for the germination of telestospores of sugarcane and *E. fulvus* rusts seems to be 20°C. Dormancy in teleutospores was noted. These teleutospores failed to infect their respective hosts indicating thereby that these rusts are not autoecious.

In a preliminary varietal resistance test out of 22 varieties three varieties, namely Co.513, Co.S.186 and Co.S.510 were found to be susceptible.

ACKNOWLEDGEMENTS : We wish to thank Dr. R. Prasada for going through the manuscript critically and helpful suggestions. Grateful thanks are also due to the then Head of the Division for providing necessary facilities for this work. Thanks are also due to Dr. Kishen Singh, Assistant Plant Pathologist in the Division at the time for miscellaneous help and assistance.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

ARTHUR, J. C. (1906) Cultures of *uredineae* in 1905. *Jour. Myc.* **12** : 11-27.

——————— (1908) Cultures of *Uredineae* in 1907. *Jour. Myc.* **14** : 7-26.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BUTLER, E. J. (1914) Notes on some rusts in India. *Ann. Mycol. Berl.* **12** : 81-82.

BUTLER, E. J. (1918) *Fungi and Disease in plants.* Thacker, Spink and Co., Calcutta.

CHONA, B. L. (1956) A brief survey of sugarcane diseases in India (Plant Pathology Section). *Proc. Int. Soc. Sug. Tech. 9th Cong.* (India).

———— (1958) Some Disease of Sugarcane reported from India in recent years. *Indian Phytopath.* **11** : 4-5.

———— AND R. L. MUNJAL (1950) *Puccinia kuehnii* (Krueg.) Butl. on Sugarcane in India. *Curr. Sci.* **19** : 151-152.

CUMMINS, G. B. (1953) The species of *Puccinia* parasitic on the *Andropogoneae uredineana* **4** : 1-90.

PATEL, J. K., M. N. KAMAT AND Y. A. PADHYA (1950) A new record of *Puccinia* on Sugarcane in Bombay. *Curr. Sci.* **19** : 121-122.

PADWICK, G. W. AND A. KHAN (1944) Notes on Indian Fungi—1. *Imp. Mycol. Inst.* Kew, **10** : 10-12.

VASUDEVA, R. S. (1956) Some Diseases of Sugarcane newly found in India. *F. A. O. Pl. Protect. Bull.* **4** : 129-131.

VAHEEDUDDIN, S., D. B. REDDY AND S. K. SAHSTRI (1955) Sugarcane Rust in Hydrabad State. *Sci. and Cult.* **21** : 328-329.

WATER, C. W. (1928) The control of teliospore experimental methods. *Phytopathology* **18** : 157-213.

YARWOOD, C. E. (1946) Detached Leaf Culture. *Bot. Rev.* **12** : 1-56.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

Serological relationship of Sunnhemp Mosaic Virus with some other Mosaic Viruses commonly occuring in Delhi;—G. P. S. ANAND AND H. S. SAHAMBI, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12.—A mosaic disease of Sunnhemp (*Crotalaria juncea* L.) has been reported from Delhi, (Vasudeva ; Raychaudhuri, Nariani and Das[7]). The disease is characterised by prominent mosaic mottling of the leaves frequently accompanied by puckering, blistering and reduction in size. The causal virus closely resembles, in the symptomatology and physical properties, the Southern Sunnhemp mosaic virus reported to be a strain of Tobacco mosaic virus from Poona by Capoor.[2]

The culture of the Sunnhemp mosaic virus and other viruses was obtained from the Culture Collection of the plant viruses being maintained in the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi. The Sunnhemp mosaic virus culture was multiplied on young sunnhemp plants raised in the insect-proof glasshouse. The mosaic affected sunnhemp leaves were processed for purification by the chemical method (to be described elsewhere) and about 2 ml. of the purified virus material was used for injection of the rabbits. The purified virus material (0.5 ml.) was mixed with an equal volume of 2N saline and injected intravenously into marginal vein of the ear of an albino rabbit. In all, four such injections were given at an interval of three days. The animal was bled fifteen days after the last injection and four such bleedings after weekly intervals yielded about 50 ml. of the clarified antiserum. The antiserum of sunnhemp mosaic virus, thus prepared, was tested against the purified virus material, as also the partially clarified sap from the mosaic infected sunnhemp leaves, and a positive precipitate was obtained. No precipitate was, however, obtained when the antiserum was tested against the sap from healthy plants.

Tests to determine the serological relationship of sunnhemp mosaic virus with some other mosaic viruses commonly occurring in Delhi were carried out with the above antiserum. Optimal dilutions in normal saline of the antiserum and the clarified sap from diseased plants of the seven plant viruses mentioned below were mixed in equal proportion and incubated at 35°C in a water bath. A flocculent precipitate was obtained with Bottle-gourd mosaic virus-BGMV (Vasudeva, Raychaudhuri and Singh[9] ; Anand, Mishra and Sahambi[1]), Tobacco mosaic virus-TMV and Cowpea mosaic virus-CPMV (Nariani and Kandaswamy[5]) indicating positive serological relationship (Table 1). However, the amount of precipitate was only slight in the case of CPMV as compared to that with BGMV or TMV. No precipitate was obtained in the case of Chilli mosaic virus (Jha and Raychaudhuri[4]), Maize mosaic virus (Chona and Seth[3]) and *Cucumis virus* 1 (Nariani and Nyako[6]), showing thereby, that none of these is related to the Sunnhemp mosaic virus.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Comparative controls set up in an identical way using clarified sap from healthy plants in each case did not give any precipitate.

TABLE 1 : Precipitin test with the antiserum of SMV against its specific antigen and some other mosaic viruses.

Serial number	Virus	Comparative amount of precipitate
1.	Sunnhemp mosaic virus (SMV)	+++++
2.	Bottlegourd mosaic virus (BGMV)	+++
3.	Tobacco mosaic virus (TMV)	+++
4.	Cowpea mosaic virus (CPMV)	++
5.	Chilli mosaic virus	—
6.	Maize mosaic virus	—
7.	*Cucumis virus* 1	—

Note : plus (+) means positive precipitation and the number of plus signs indicates comparative amount of precipitate.
minus (—) means no precipitate.

ACKNOWLEDGEMENTS : The authors are thankful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his keen interest during the course of the work and also to Mr. T. K. Nariani, Virus Pathologist, for critically going through the manuscript.

[1]Anand, G. P. S., M. D. Mishra and H. S. Sahambi. *Indian J. Microbiol.* **1** : 181-183 (1961).

[2]Capoor, S. P. *Phytopathology* **52** : 393-397 (1962).

[3]Chona, B. L. and M. L. Seth. *Indian J. agric. Sci.* **30** : 25-30 (1960).

[4]Jha, A. and S. P. Raychaudhuri. *Indian J. agric. Sci.* **26** : 217-222 (1956).

[5]Nariani, T. K. and T. K. Kandaswamy. *Indian Phytopath.* **14** : 77-82 (1961).

[6]Nariani, T. K. and K. O. Nyako. *Indian Phytopath.* **16** : 313-316 (1963).

[7]Raychaudhuri, S. P., T. K. Nariani and C. R. Das. *Indian Phytopath.* **15** : 79-80 (1962).

[8]Vasudeva, R. S. Annual Scientific Reports of the Indian Agricultural Research Institute for the year ended 30th June, 1952 : 84 (1952).

[9]Vasudeva, R. S., S. P. Raychaudhuri, and J. Singh. *Indian Phytopath.* **2** : 180-185 (1950).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Preliminary Biochemical Observations of Leaf Spot Disease of Groundnut—V. RAVINDRA NATH, L. G. KULKARNI AND B. APPAJI RAO., Regional Research Centre, 'Pirrcom' Hyderabad-30—Disease resistance has been related to contents of certain chemical constituents like riboflavin[1], ascorbic acid[2] and magnesium[3]. In the present study investigations were carried out on the quantitative build up ascorbic acid, riboflavin, and chlorophyll, at various growth stages as related to the resistance and susceptibility of varieties.

Two varieties T. M. V. 2 and T. M. V. 3 were selected. Composite samples were drawn at comparable physiological stages only.

The data given in table shows that in the susceptible variety there is an increase of ascorbic acid and the highest intensity of disease is at maturity. This is more pronouned in diseased than in healthy samples. In the moderately resistant variety T. M. V. 3 there is a decrease in both the samples. It is also seen that in T. M. V. 2 the disease increased with age starting from flowering to maturity. Normally in groundnut the disease development is more between pod formation. In the diseased leaves it is probable that the enzymic oxidation of Ascorbic acid is disorganized resulting in acid accumulation. In T. M. V. 3 such sharp differences are not noticeable due to low intensity of disease. These results are in conformity with Puspanadem.[2]

The riboflavin content in healthy samples is much higher than in diseased samples of T. M. V. 2 and T. M. V. 3. This is very clear at maturity. In T. M. V. 3, at pod formation also, the riboflavin in healthy samples is nearly 4.5 times that of diseased samples (875.0 to 192.5 microgram). This would indicate that riboflavin may be playing an important role in developing resistance to Tikka disease. This is partly corroborated by Miller.

The chlorophyll analysis indicates that it generally decreased with advance in age. In diseased samples of T. M. V. 2 the fall is very steep. It is significant that T. M. V. 3 with darker green leaves should contain less total chlorophyll at pod formation than T. M. V. 2 where they are thinner and light green.

These investigations have shown that ascorbic acid appears to have a direct bearing on 'TIKKA' disease in susceptible variety and that higher riboflavin content of leaves is associated with resistance. It appears that the two varieties have a differing pattern of variation in chlorophyll. Though the moderately resistant variety T. M. V. 3 has a lower initial chlorophyll content than susceptible variety the same amount was found at maturity whereas susceptible variety recorded only 50 per cent of the original (i. e. 129/257 mg.).

ACKNOWLEDGEMENTS : Grateful thanks to Dr. P. G. Tulpule of the Nutrition Research Laboratories, Hyderabad and Dr. A. Appa Rao of the Agriculture College, Hyderabad.

---

[1]Miller L. I. Ph. D. Thesis, University of Minnesota, 1953.

[2]Puspanadem, P. *Oryza sativa. Curr. Sci.* 26 : 1 26, 1957.

[3]Bledsoe, R. W., H. C. Harris and W. B. Tisdale. *Plant. Physiol.* 21 (2) : 237-240 (1964).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE : Showing ascorbic acid chlorophyll and riboflavin content (Results on fresh weight basis and mean of quadruplicate determination).

Stage of crop	Ascorbic Acid Miligrams/100 grams				Chlorophyll (mg/100 grams)				Riboflavin (Micrograms/100 grams)				Tikka Disease intensity T. M. V. 2
	T. M. V. 2		T. M. V. 3		T. M. V. 2		T. M. V. 3		T. M. V. 2		T. M. V. 3		
	Diseased sample	Disease free	Diseased sample	Disease free	Diseased sample	Disease free	Diseased sample	Disease free	Diseased sample	Disease free	Diseased sample	Disease free	
Flowering	33.8	35.9	22.0	75.9	921.9	257.4	162.4	153.7	—	175.00	350.00	175.00	0.3
Pod formation	22.2	60.5	11.3	12.3	165.4	212.2	209.0	175.7	332.0	—	192.5	875.00	3.5
Maturity	111.8	38.9	22.4	30.0	237.9	129.2	147.8	158.8	280.0	315.00	420.00	770.00	21.9

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Some important observations on the staining of Bacterial Flagella with modified Bailey's method :—Y. P. RAO AND V. K. MATHUR*, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—The number and arrangement of flagella, in case of plant pathogenic bacteria, greatly aid in their identification upto genera. In general, several methods viz., Casares-Gil Flagella stain as published by Plimmer and Paine[5], Gray's[3], Leifson's[4] and Bailey's[1] modified by Fisher and Conn[2] have been recommended for demonstrating bacterial flagella, but, there has always been some difficulty in staining these fibrils by most of these methods. The modified Bailey's method is one of the most commonly used and according to Fisher and Conn[2] this method is a rapid and constant one for routine flagella staining of all motile aerobic organisms. The following staining solutions are employed in this method :

Solution I

Tannic acid (10 per cent aqueous solution)	...	18 ml.
Ferric chloride (6 per cent aqueous solution)	...	6 ml.

Solution II

Solution I	...	3.5 ml.
Basic fuchsin (0.5 per cent in ethyl alcohol)	...	0.5 ml.
HCl, concentrated	...	0.5 ml.
Formalin	...	2.0 ml.

Solution III

Ziehl's Carbol Fuchsin

Solution A

Basic fuchsin (90 per cent dye content)	...	0.3 g.
Ethyl alcohol (95 per cent)	...	10 ml.

Solution B

Phenol	...	5 g.
Distilled water	...	95 ml.

Mix solution A and B.

Fisher and Conn[2] suggested that solutions I and II should be prepared fresh each time and used as many times as desired in any one day, but must not be kept overnight. Several attempts in the past have been made in this laboratory to stain flagella of plant pathogenic bacteria by following Bailey's modified method but the results were never satisfactory even after observing all the necessary precautions such as cleanliness of the microslides, preparation of the smears, etc.[6] The flagella were either faintly stained or not at all.

*Present address : Division of Entomology, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Recently, attempts to stain flagella of an insect pathogen (*Pseudomonas* sp.) were also not encouraging. Incidentally, when the bacterial smears were stained with stains (3 days old) instead of freshly prepared ones the flagella were readily stained. This suggested that ripening of staining solutions has got something to do with this success. The solution II which was dark-blue in fresh preparations, showed some blue sedimentation with light to dark brown supernatent liquid after 24 hours, while the other two solutions showed no noticeable change in them. A detailed experiment was, therefore, carried out to confirm the observation and also to study the differential ability of fresh and old stains in staining bacterial flagella. The staining solutions were prepared in large quantities and preserved in tightly rubber-stoppered flasks. As many as sixty bacterial smears, each of an insect pathogen (*Pseudom onas* sp.), red stripe of sugarcane organisms (*Pseudomonas rubrilineans* and *Xanthomonas rubrilineans*, respectively) and blight pathogen of rice (*Xanthomonas oryzae*), were prepared from 24 hours old actively growing cultures, taking all the precautions. The same method was employed in staining the smears.

It was found that flagella were stained either faintly or not at all when fresh strains were used. The staining property of the solutions gradually increased with the age upto 10 days and thereafter it decreased once again.

In another experiment, to find out which of the three solutions needs aging, two of the staining solutions were kept fresh at a time and the third being three days old. It was found that all the three solutions require ripening to be effective.

Thus, it is concluded that the ripening of the staining solutions used in the modified Bailey's method is very necessary to successfully stain plant and insect pathogenic bacterial flagella.

The authors are grateful to Dr. B. L. Chona, the former Head of the Division of Mycology and Plant Pathology for his keen interest in the work and for the facilities provided. Our grateful thanks are also due to Dr. D. N. Srivastava, Plant Bacteriologist, for his valuable suggestions.

---

[1]Bailey, H. D. *Proc. Soc. Exptl. Biol. Med.*, **27** : 111-112, (1929).

[2]Fisher, P. J. and Jean E. Conn. *Stain Technol.*, **17** : 117-121, (1942).

[3]Gray, P. H. H. *J. Bacteriol.*, **12** : 273-274, (1929).

[4]Leifson, Einar. *J. Bacteriol.*, **20** : 203-211, (1930).

[5]Plimmer, H. G., and S. G. Paine. *J. Pathol. Bacteriol.*, **24** : 286-288, (1921).

[6]Society of American Bacteriologists, *Manual of Microbiological Methods*, 305 pp., (1957).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Notes on Powdery Mildews from India-III.—G. LALL AND J. N. GUPTA,—Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12.—Amongst the Mycological collections from the Kulu-Manali Hills, Punjab, a few interesting specimens of Powdery mildews were discovered, which are reported in this paper. The specimens have been deposited in the Herbarium Cryptogamae Indiae Orientalis, I. A. R. I., New Delhi.

I. *Erysiphe cichoracearum* DC. em. Salmon in Mem. Torrey bot. Cl., 9 : 163, 1900 ; Blumer in Beitr. Krypt. Flora Schweiz, 7 (I) : 246, 1933.

On living leaves of *Physochlaina praealta* Hk. f. (=*Hyoscyamus praealtus* Walp.), (Solanaceae), Manali, Kulu, Punjab, 6-8-1963, V. S. Sharma, H. C. I. O. No. 28518 (Fig. I).

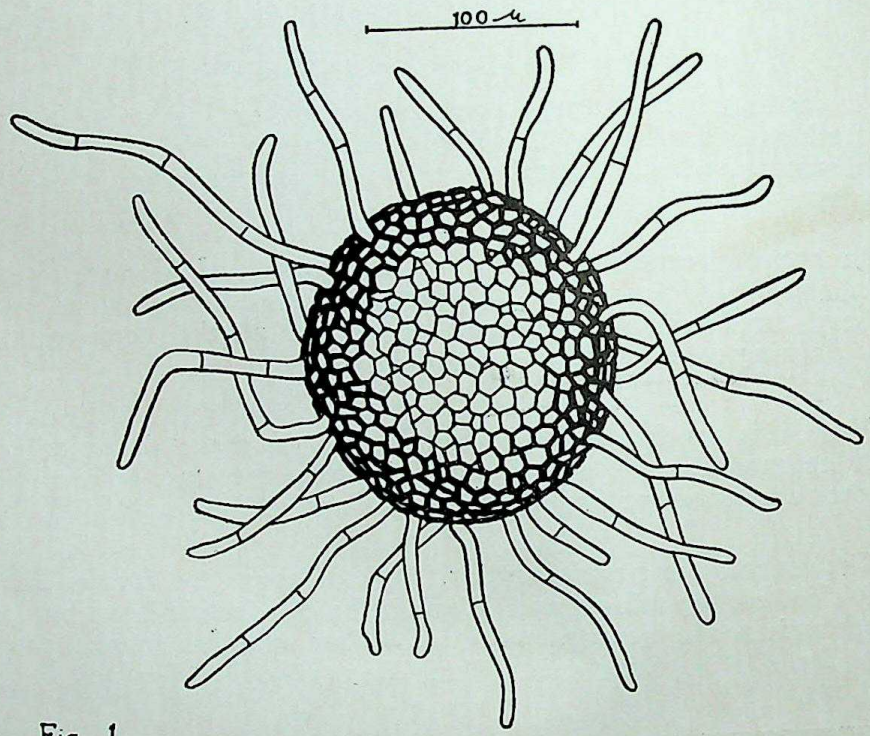
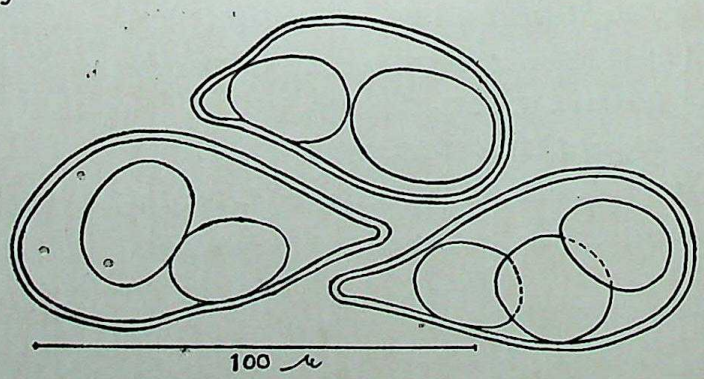


Fig. 1



CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

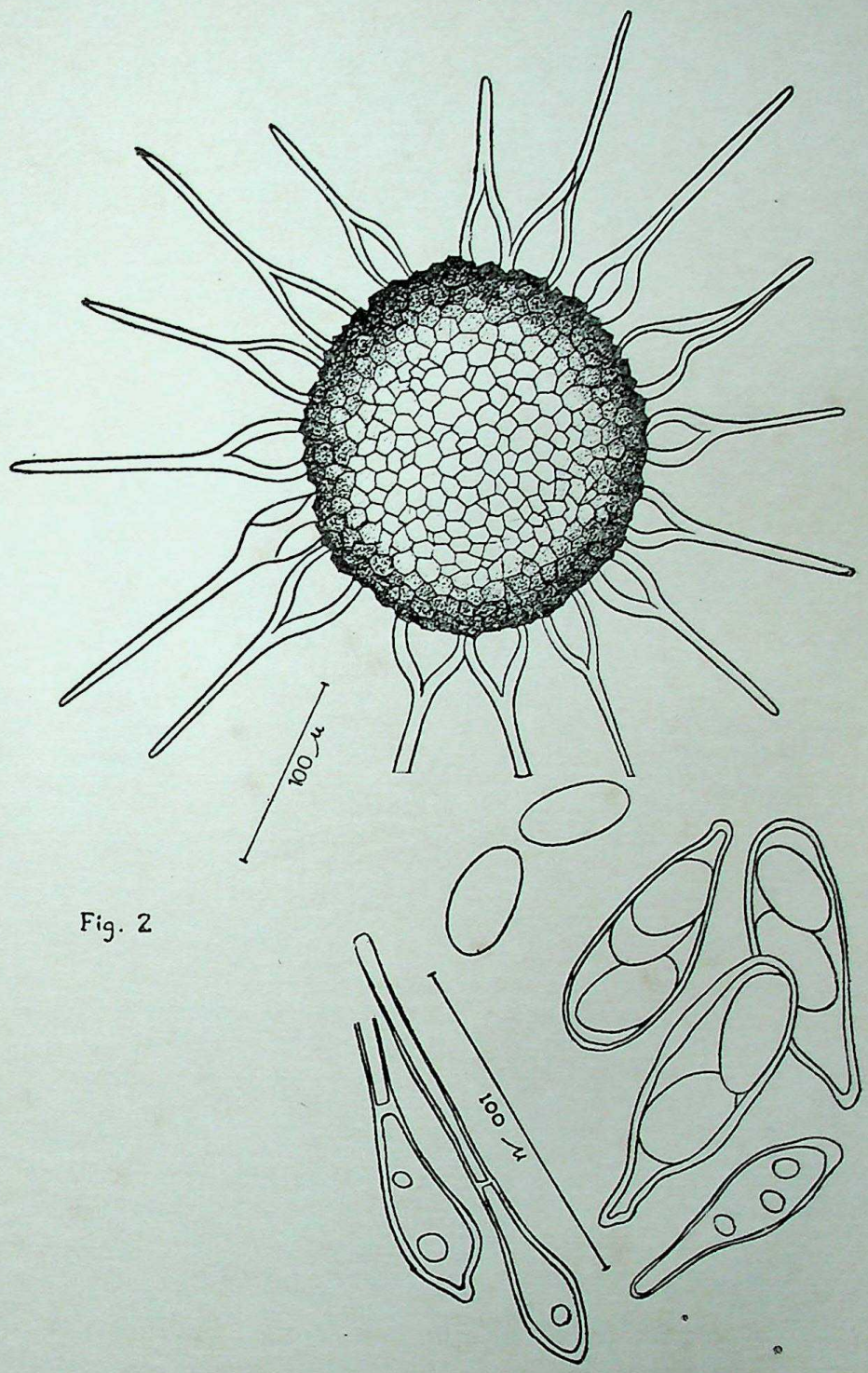


Fig. 2

The powdery mildew on this host is inconspicuous. Mycelium is evanescent and no conidia are observed. It is usually hypophyllous. Cleistothecia are uniformly scattered. They are globose-depressed and are 100-140 μ in diameter. Appendages are numerous, brown and are

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

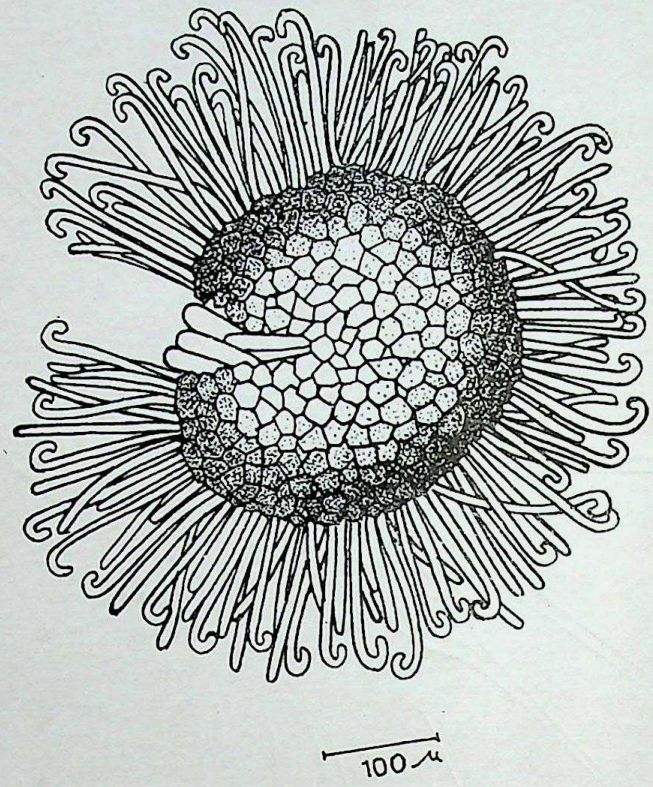
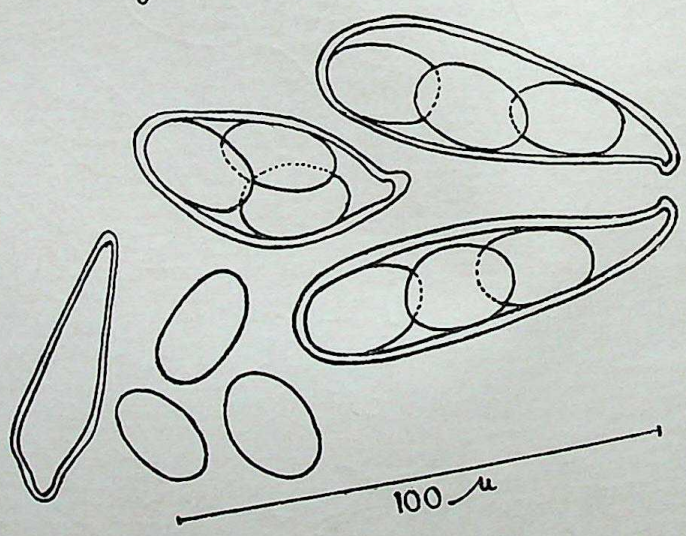


Fig. 3



of variable length. Asci are usually two-spored, but occasionally 3-spored asci have also been observed. They measure 56-80 × 32-44 μ. The ascospores are ovate to subglobose, 22-36 × 20-30 μ in size.

Earlier, it was recorded on *Hyoscyamus niger* L., from Germany, Bulgaria, Russia and Yugoslavia. This host genus is being reported for the first time from India.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

2. *Phyllactinia suffulta* (Rabenh.) Sacc., in Syll. Fung., **1** : 5, 1882.

On living leaves of *Cedrella toona* Roxb. (Meliaceae), Palampur, Punjab, 31-1-1964, V. S. Sharma, H. C. I. O. No. 28517 (Fig. 2).

It produces inconspicuous patches of varying sizes on the under surface of the leaf. Mycelium is evanescent. Conidia are of *Ovulariopsis* type, and measure 48-60 × 16-20 μ. Cleistothecia are uniformly scattered and are 200-260 μ in diameter. Appendages are up to 16, variable in size, 40-300 μ in length, hyaline to subhyaline, rigid, with a swollen base and blunt and pointed apex. Asci are 2-3 spored and measure 60-90 × 32-40 μ. Ascospores are ovate, hyaline with dense protoplasmic contents and measure, 28-36 × 18-22 μ.

This species has a very wide host range, to which the above reported host is a new addition.

3. *Pleochaeta shiraiana* (Henn.) Kimbr. and Korf in Mycologia, **55** : 624, 1963.

Syn. Uncinula shiraiana Henn., in Engler's Botan. Jahrb., **29** : 148, 1900.

On living leaves of *Celtis* sp., Palampur, Punjab, 30-1-1964, V. S. Sharma, H. C. I. O. No. 28519 (Fig. 3).

It produces whitish patches of varying sizes on the lower surface of the leaf. Mycelium dense. Conidia are of *Ovulariopsis* type and measure 48-56 × 12 μ. Cleistothecia are gregarious or scattered, globose to subglobose and measure 243-283 μ. Appendages are very numerous, crowded, smooth, thick-walled, simple, hyaline with uncinate apex. Asci are numerous, fusiform-elliptical, rarely oblong, stalked, and measure 88-100 × 24-28 μ. Ascospores are always 3, and measure 22-29 × 14-18 μ.

The earlier collections from India (*Celtis australis* Linn. = *C. caucasica* Willd.), Harwan, Kashmir, 10-8-1906. E. J. Butler, H. C. I. O. No. 942 ; *C.* sp., Kulu, Punjab, 29-11-1957, H. S. Gill, H. C. I. O. No. 25901, reported as *Uncinula polychaeta* (Berk. and Curt.) ex. Ellis, in Butler and Bisby. (The Fungi of India, I. C. A. R., Sci. Mono. No. **1** : 40, 1931) on re-examination were found to belong to *P. shiraiana*. This confirms Kimbrough and Korf (l. c.) finding that all asiatic collections belong to *Pleochaeta shiraiana*.

The authors are grateful to Dr. J. N. Kapoor, Herbarium Keeper, for suggestions and help. Sincere thanks are due to Dr. B. L. Chona, former Head of the Division of Mycology and Plant Pathology, for his keen interest and encouragement and also to Shri B. P. Agarwal, artist, for help in the line drawings.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Natural occurrence of specialised forms of Puccinia graminis and P. striiformis on Lolium perenne.– D. P. MISRA, S. T. AHMAD AND SHEODHAN SINGH, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—*Lolium perenne* is an introduced grass in Simla hills which now grows wild.[1] During the course of surveys in and around Simla, it was found to be infected with two rusts—identy based on colour and size of uredo pustule. Morphological studies revealed that one of the rusts resembles with that of *Puccinia graminis* and the other with *P. striiformis*.

P. graminis and *P. striiformis* have been reported to comprise numerous specialised forms infecting various grasses. Some of these grasses also harbour the specialised forms of the rusts infecting cereals like wheat and oat and as such serve as collateral hosts. Different forms of *P. graminis* on *Agropyron semicostatum* and *Poa nemoralis* and of *P. striiformis* on *A. semicostatum Phalaris minor* and *Muehlenbergia hugelii* have been reported from India and it has been further established that *A. semicostatum* and *M. hugelii* function as collateral hosts of black rust and yellow rust respectively[4, 5]. Besides these, several other indegenous and exotic grasses have been reported to get infected with *P. graminis tritici*, *P. g. avenae* and *P. striiformis* in laboratory or in nature [2, 3, 5, 6, 7].

There appears to be no record of any rust on *L. perenne* in the country except that collections of this grass received from Australia and Pakistan (Quetta) when grown at Delhi during 1948-49 were found to be infected with *P. g. tritici*[6]. However, this grass has been reported to be host for both, *P. graminis* and *P. striiformis* from elsewhere but no pathological tests seem to be attempted to ascertain that the forms of these rusts are specialised on *L. perenne*.

The two rusts were maintained on the original host and their pathogenicity was studied under glasshouse conditions. It was found that the rusts are restricted to the original host, whereas eleven exotic collections of *Lolium* sp. (E. C. 16005, E. C. 16127, E. C. 16473, E. C. 16746, E. C. 16747, E. C. 16752, E. C. 16753, E. C. 16754, E. C. 18330, E. C. 18332 and E. C. 20647), eight exotic collections of *Phleum* sp. (E. C. 16734, E. C. 16738, E. C. 16739, E. C. 17946, E. C. 17947, E. C. 18333, E. C. 21246 and E. C. 21247) nine exotics of *Festuca* sp. (E. C. 15123, E. C. 16733, E. C. 16741, E. C. 16745, E. C. 19749, E. C. 17037, E. C. 17948, E. C. 18329 and E. C. 18342) two indegenous *Festuca* spp. (I. W. 1849 and I. W. 1850), exotic *Avena elatior* (E. C. 18339) indeginous *Agropyron semicostatum*, *Helictotrichon virens*, *Poa nepalensis*, *Muehlenbergia hugelii*, *Triticum aestivum* (var. Agra local) and *Hordeum vulgare* (var. Agra local) did not get infected. On the other hand the plants of *Lolium perenne* from Simla were found to be immune to the races of *P. graminis tritici* and *P. striiformis* of wheat, in separate mixtures.

In the light of this, it is concluded that the rusts herein reported are specialised forms of *Puccinia graminis* and *P. striiformis* on *Lolium perenne*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. L. Chona, former Head of the Division of Mycology and Plant Pathology, I. A. R. I., New Delhi for his encouragement and to Dr. R. Prasada, for his helpful suggestions. Thanks are also due to Shri. S. A. Dadlani, Division of Plant Introduction, I. A. R. I., Simla for providing material of grasses.

---

[1]Collett, H. *Thacker Spink* and co. (1921).
[2]Lele, V. C. and M. H. Rao. *Indian Phytopath.* **14** : 154-159 (1961).
[3]Mehta, D. P. *I. C. A. R. Mongr.* (1940).
[4]Misra, D. P. and V. C. Lele, *Indian Phytopath.* **16** : 382-384 (1963).
[5]Prasada, R. *India J. Agric. Sci.* **18** : (1948).
[6]——————— *Curr. Sci.* **20** : 243, (1951).
[7]Vasudeva, R. S., L. M. Joshi and V. C. Lele, *Indian Phytopath.* **6** : 39-46 (1953).

———

Relative Efficacy of Fungicides for the Control of Cladosporium variabile causing Leaf Spot Disease of Spinach-(*Spinacia oleracea*)—R. L. MATHUR AND S. P. SEHGAL—Plant Pathology Section, Rajasthan, Jaipur—Leaf spot disease of wrinkled variety of Spinach (*Spinacia oleracea* L.) caused by *Cladosporium variabile* is very commonly found in winter months in Rajasthan as reported by Mathur, Mathur and Sehgal (1959)[2]. Since the disease results in final drying of leaves and defoliation, the losses are apparent as the vegetable is grown for leaves only. Experiments to determine the relative efficacy of foliar fungicides containing different chemicals as active ingredients in checking the disease spread were conducted in greenhouse. The fungicides tried were—1.00 per cent Bordeaux mixture, 0.25 per cent Blitox -50 (Copper oxychloride), 0.25 per cent Crag fungicide—658 (Copper Zinc chromate) and 0.25 per cent Fungimar (Cuprous oxide).

Seeds of wrinkled variety of Spinach were grown in 9″ pots and 10 plants in each pot with three replications were used for each fungicidal treatment. On six week old plants, the stock solution of fungicides were sprayed with an atomizer at 100 gallons per acre (4.5 ml. per pot) and after 24 hours, inoculation with a heavy spore suspension from 21 days old fungus culture was carried out. In another set, fungicidal treatment was given twice i. e. after 6 weeks and 8 weeks of sowing. Two sets, one inoculated and other uninoculated with no fungicidal treatment were kept as control. The pots were kept in moist chamber for 48 hours and then placed in pot house.

The number of leaves were counted at the beginning of the experiment and those drying due to the spots were noted after two and three weeks of inoculation. After four weeks, every leaf was examined and the number showing drying and leaf spots were recorded finally. The total infection was calculated as the number of leaves dried plus 50 per cent of those showing leaf spots ; this number divided by the total

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

number of leaves and multiplied by hundred gives the percentage infection. The percentage efficiency of disease control of different fungicides was calculated by the following formula (Chester, 1950)[1].

$$\text{Per cent efficiency} = \frac{\text{Per cent infection in control} - \text{Per cent infection in treatment}}{\text{Per cent infection in control}} \times 100$$

Detailed observations as recorded in the experiment for different treatments are presented in Table 1.

TABLE 1 : Table showing the incidence of leaf spot disease (*Cladosporium variabile*) of Spinach after different fungicidal treatments in pots.

S. No.	Treatment	No. of sprays	No. of leaves Total (3 pots) (a)	No. of leaves Killed (b)	Showing leaf spots (c)	Per cent infection $\frac{(b+\frac{1}{2}c)}{a} \times 100$	Percentage efficiency for disease Control
1.	Bordeaux mixture (5 : 5 : 50)	1	260	13	20	8.85	82.9
2.	,,	2	315	10	15	5.55	89.2
3.	Blitox-50 (0.25 per cent)	1	263	87	61	44.68	13.7
4.	,,	2	258	43	32	22.87	55.8
5.	Crag fungicide-658 (0.25 per cent)	1	323	45	27	18.11	65.0
6.	,,	2	331	28	23	11.93	76.9
7.	Fungimar ,,	1	287	21	27	12.02	76.7
8.	,,	2	317	21	18	9.49	81.5
9.	Control A. (Inoculated)		251	116	28	51.79	
10.	Control B. (Uninoculated)		217	0	0	0	

From the figures of the percentage efficiency of disease control it is apparent that Bordeaux mixture is the best fungicide out of the four under trial. Two sprayings in each case showed better control of the disease than one. Fungimar comes next in the merit closely followed by Crag fungicide--658.

The authors are highly grateful to Dr. N. Prasad, Dean, S. K. N. college of Agriculture, Jobner for his valuable guidance and to the Director of Agriculture for providing facilities.

[1]Chester, K. S. *Plant Dis. Reptr.* Supplement 193, 1950.

[2]Mathur, R. L., B. L. Mathur and S. P. Sehgal. *Phytopathology* **12** : 161-163. (1959).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A Fusarium sp. with Cellulolytic and Lignolytic properties from manure pit—G. RANGASWAMI AND P. RAJASEKARAN,—Microbiology Laboratory, Faculty of Agriculture, Annamalai University, Annamalainagar, Madras.—In an attempt to isolate fungi with combined cellulolytic and lignolytic properties from sewage, soils and manure pits, only a single species of *Fusarium* isolated from a manure pit in the University Experimental Farm, was found to possess the combined activity on cellulose and lignin, while several others were only cellulolytic in varying degrees of efficiency. In the farm compost there were 2.3 million bacteria, 0.2 million fungi and 1 million actinomycetes per gm. of manure on dry weight basis of which 1.2 million bacteria, one thousand fungi and 24 thousand actinomycetes were cellulolytic. When selected isolates of the cellulolytic fungi were examined in culture media one out of the several hundreds tested was found to be lignolytic.

TABLE 1 : A comparison of the cellulolytic and lignolytic properties of *Fusarium* sp. in modified Czapek's medium

	Reduction of cellulose	Reduction of lignin
Maximum reduction under static conditions in liquid medium	53.0 per cent	6.3 per cent
No. of days taken for maximum reduction under static conditions	19	27
Maximum reduction under submerged aerated conditions in liquid medium	38.7 per cent	5.9 per cent
No. of days taken for maximum reduction under aerated conditions	23	27
Optimum pH of the substratum for reduction	6.0	7.0 to 8.0
Best nitrogen source for reduction	Sodium nitrate (0.5 per cent nitrogen)	Ammonium sulphate or urea (0.5 per cent nitrogen)
Influence of riboflavin (0.01 per cent) on reduction	No change	Increased action

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The fungus *Fusarium* sp., was isolated on modified Czapek's agar with cellulose as the only carbon source and was then examined for lignolytic property in modified Czapek's agar containing 2 per cent lignin. Lignin from saw-dust, prepared by the method of Klason[3], was used in these studies. The reduction percentages of cellulose and lignin due to the fungal activity were obtained by growing them in 75 ml. of modified Czapek's solution with 2 per cent pure cellulose or lignin in 250 ml. Erlenmeyer flasks and estimating the cellulose or lignin left over in the medium, at various time intervals. The procedures followed for the purpose were the same as detailed by Rangaswami and Ramabadran[6]. Some of the cultural conditions influencing the activity of the fungus were studied and the results are summarized in Table I.

The organism does not seem to favour the submerged aerated conditions, on wrist action shaking machines, for the reduction of cellulose and lignin, as compared to the static conditions. Among the nitrogen sources tested, sodium nitrate was the best for cellulose reduction and ammonium sulphate and urea equally favourable for lignin reduction. Riboflavin enhanced lignolytic activity of the fungus but not the cellulolytic activity. The cellulolytic property of *Fusarium* spp. has already been reported by Garrett[1], Hussain and Dimond[2], Pugh *et al.*[4] Rangaswami and Chandrasekaran, Reese and Levinson[7] and Venkat Ram[8] but this is the first report on the combined cellulolytic and lignolytic activity of an isolate of *Fusarium*.

---

[1]Garrett, S. D. *Trans. Brit. mycol. Soc.* **46** : 572-575 (1963).

[2]Hussain, A. and A. E. Dimond. *Proc. Nat. Acad. Sci.* **44** : 594-601 (1958).

[3]Klason, P. *Cellulosche*me, **4** : 81 (1923).

[4]Pugh, G. J. F., J. P. Blakeman, M. G. Jones and H. O. W. Eggins. *Trans. Brit. mycol. Soc.* **46** : 565-571 (1963).

[5]Rangaswami, G. and A. Chandrasekaran (1963) *Indian J. Microbiol.* **3** : 35-40 (1963).

[6]Rangaswami, G. and R. Ramabadran (1963). *Indian J. Microbiol.* **3** : 139-142 (1963).

[7]Reese, E. T. and H. S. Levinson. *Physiol. Planta* **5** : 345-366 (1952).

[8]Venkat Ram, C. S. *Proc. Nat. Inst. Sci., India* **22** : 204-211 (1956).

———

Anthracnose of Celosia argentea var. Cristata in Delhi—R. L. MUNJAL AND J. N. GUPTA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—*Celosia argentea* Linn. var. *Cristata* Linn. (Cock's comb) is a common ornamental plant grown by floriculturists. It flowers during rainy weather i.e. July-September but continues till the end of December.

During October-November, 1964, a serious disease on this plant was observed at Delhi, as a result of which a large number of plants died. The characteristic symptom of the disease (Fig. 1) is the formation of water soaked, later straw coloured lesions on the stem or branches, which bear a large number of black dot like acervuli. Under humid conditions,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

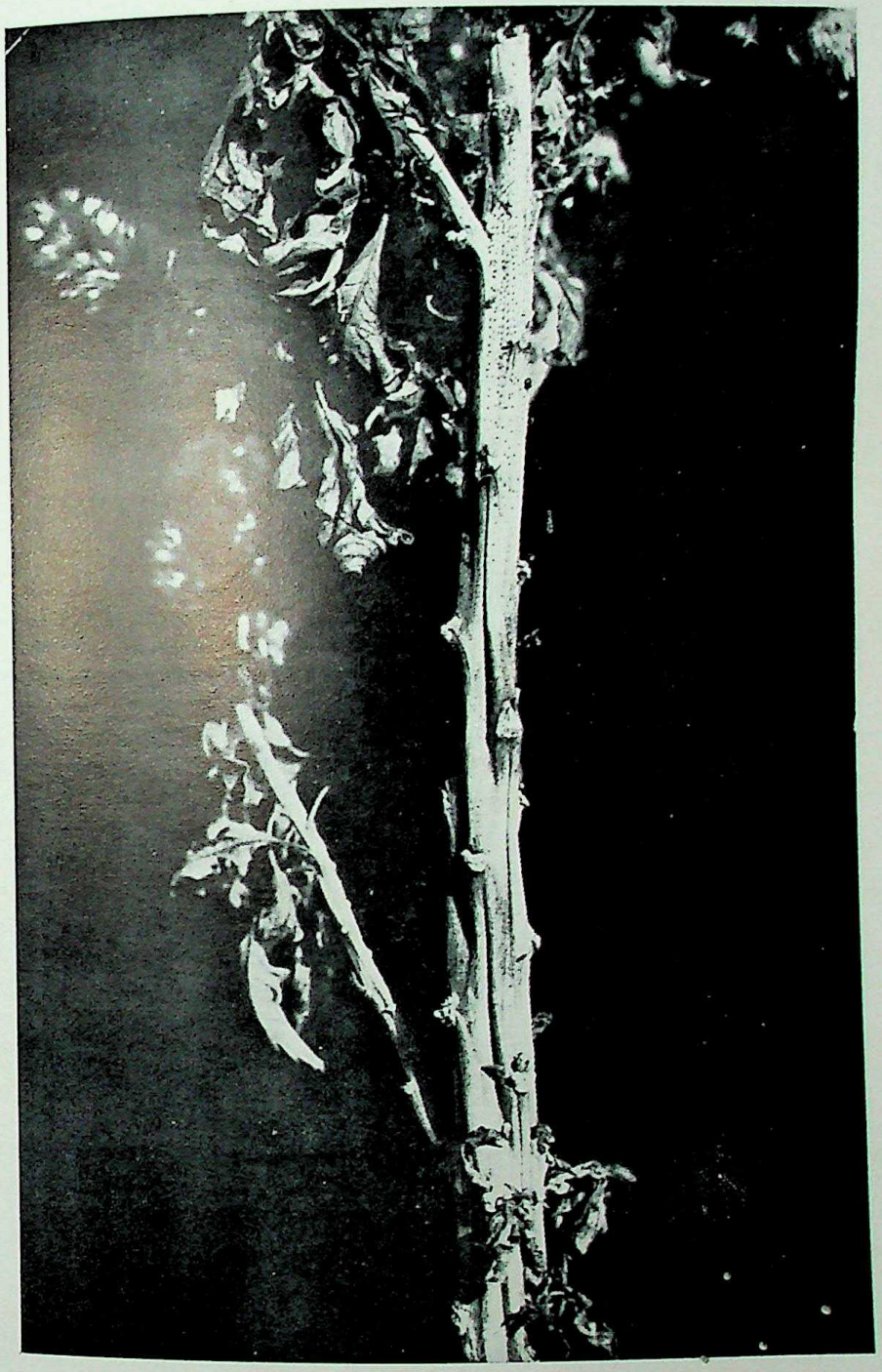


Fig. 1. Diseased stem of Cocks' comb showing black dot like acervuli.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

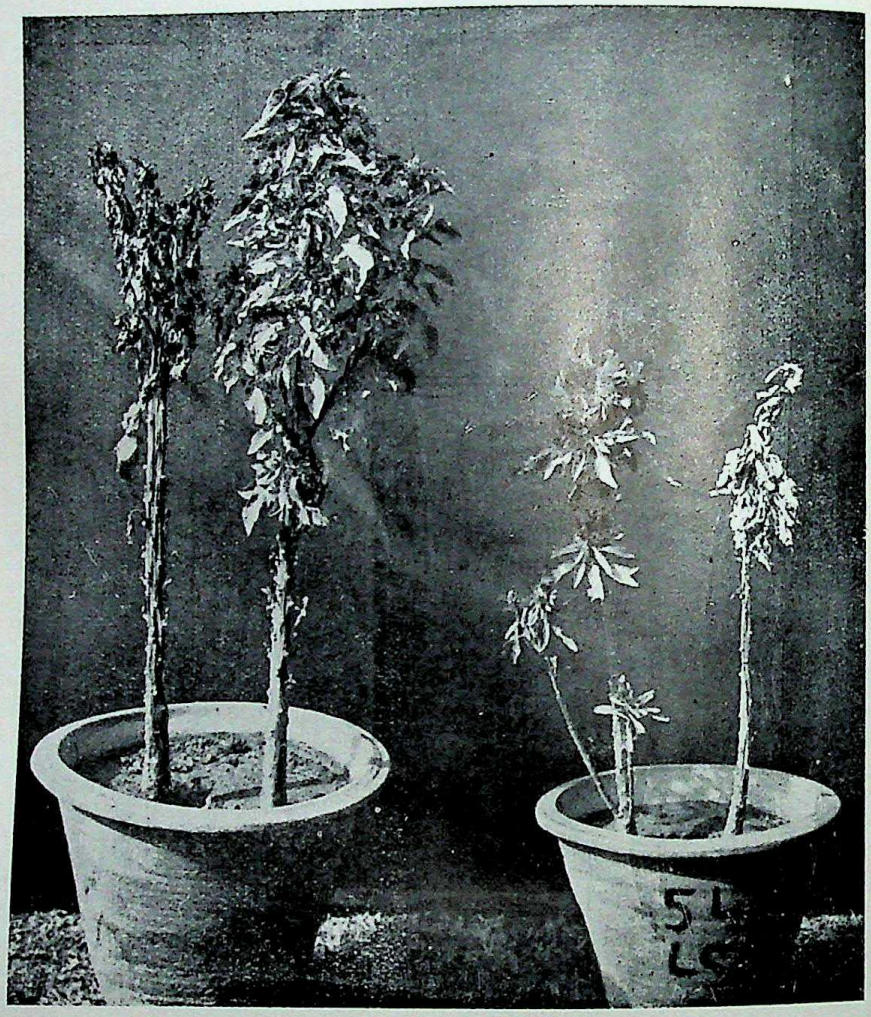


Fig. 2. One diseased and one healthy plants of Cocks' comb in each plot.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

a pinkish ooze comes out of the acervuli that imparts a pinkish colour to the lesion. The lesions vary in size considerably from 2-20 cm. in length and 0.5—2.5 cm. in breadth. The affected portion of the plant becomes shrivelled and leaves above this portion of the stem or branch start withering. They never recover again and the whole shoot or the plant completely dries up (Fig. 2). Sections of the affected tissue upon microscopic examination show the presence of sep ate, hyaline or sub-hyaline intracellular hyphae 2.5-3.5 μ thick. The cells of the diseased tissue lose their shape and are somewhat brownish in colour. Later hyphae collect underneath the epidermis at certain places, to form a mat like stroma of small irregular fungal cells. This is olivaceous in colour and 5-6 layer thick. A close compact layer of hyaline single celled cylindrical or phial-shaped conidiophores measuring 8-10 $\times$ 4-5 μ in size is produced on the stroma. On each conidiophore develops an elongated elliptic, hyaline single celled conidium with a thin wall and granular protoplasmic contents, measuring 12-16 $\times$ 5-6 μ. The conidia form a slimy pinkish mass when the acervuli are mature. The acervuli measure 180-200 μ in diameter. Occasionally dark brown setae, which are septate and pointed towards the apex may also be observed arising in between the conidiophores.

Isolations made by plating the surface sterilized diseased tissues on plain agar and incubated at (20-25°C) room temperature yielded a fungus, which was further purified by single spore technique by growing over Potato Dextrose agar. In culture, the fungus forms a whitish mat of creeping hyphae on the agar surface with little or no aerial mycelium and later turns as smoke-gray. The acervuli start as aggregate of dark brown hyphal masses on which are produced typical spores (conidia) of the fungus as observed on the natural host. The spores germinate readily in tap water after 4-6 hours at room temperature. They become bicelled and produce germ tubes from both ends and very soon form appressoria. Of the three media tried viz. Oat meal agar, Czapek's agar and Potato Dextrose agar (PDA), the fungus grew best on PDA at 25°C. At 35°C or 15°C, the growth was very poor and sporulation almost completely suppressed, though the fungus could withstand these temperatures for a long time.

Pathogenicity tests were conducted with the pure cultures of the fungus by adding the inoculum to soil or by applying the culture on the stem, with or without wounds. The infection started early in the wounded tissue though ultimately there was no difference in wounded or unwounded ones. Soil infection was not successful. Typical lesions of the disease were observed on the 5th day. which gradually increased in size. Reisolations from the diseased tissues yielded the same fungus. The disease is favoured by mild, damp weather.

The causal fungus has been identified as *Colletotrichum gloeosporioides* Penz. This disease is being reported on this host for the first time.

Sincere thanks are due to Dr. B. L. Chona, Head of the Division for his keen interest and encouragement.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Occurrence of Tristeza Virus in Citrus in Northern India :—T. K. NARIANI, H. S. SAHAMBI AND B. L. CHONA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi :— Vasudeva and Capoor[2] reported the presence of Tristeza virus in the die-back affected mosambi orange (*Citrus sinensis* Osbeck var. Mosambi) trees in the Maharashtra State. Later, Capoor[1] found that Indian *Kagzi* lime [*Citrus aurantifolia* (Christm.) Swing.] was also a very good indicator plant for the virus in addition to the West Indian Key Lime. In the present note the occurrence of the virus in Northern India is being reported for the first time.

During the routine survey and indexing of suspected Citrus trees affected with die-back symptoms, bud-wood collected from Malta [*Citrus sinensis* (L.) Osbeck] var. Jaffa and Mosambi (*C. sinensis* var. Mosambi) from the orchard in the Horticulture Division of the Indian Agricultural Research Institute was grafted on 1-1½ year old seedlings of *Kagzi* lime raised from seed in the Insect-proof glass-house at Delhi during the month of February, 1964. By September-October, 1964, the young leaves in the new growth of the budded plants started showing vein-clearing followed by chlorotic mottle typical of the tristeza virus (Fig. 1), thus indicating the presence of tristeza virus in them. Further work on indexing of other Citrus species is in progress.

Grateful thanks are due to U. S. D. A. for kindly providing the funds for carrying out this work under a PL 480 Scheme for Die-back disease of Citrus in India.

---

[1]Capoor, S. P. *Indian Phytopath.* **14** : 109-112, (1961).

[2]Vasudeva, R. S. and S. P. Capoor., *FAO Plant Prot. Bull.* **6** : 91, (1958).

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

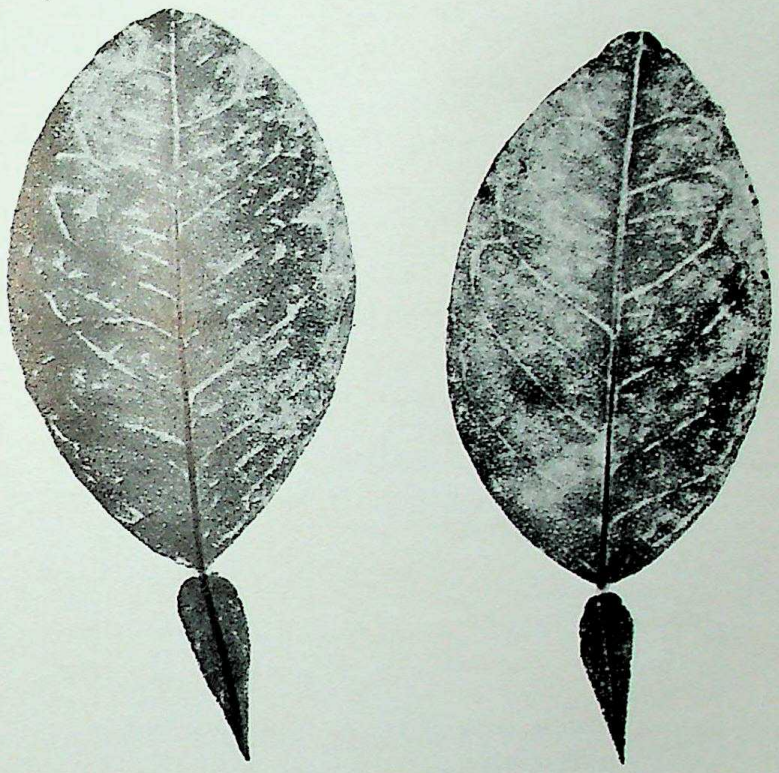


Fig. 1. Leaves of Kagzi lime (*Citrus aurantifolia* (Christm.) Swing.) showing vein-clearing and chlorotic mottle typical of tristeza virus when grafted with bud-wood from die-back affected Sweet Orange (*Citrus sinensis*) (L.) Osbeck) trees.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

a

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REVIEWS

Virus Diseases of Cotton by S. A. J. Tarr. pp 1-23. Miscellaneous Publication No. 18 issued by Commonwealth Mycological Institute, Kew, Surrey, England. Price 7s. 6d.

The publication describes eleven virus diseases of cotton reported from different parts of the world. The author has discussed in the light of his experiences as an investigator of Cotton diseases at Sudan, the possibilities of transmission of the diseases to other malvaceous hosts, varietal susceptibility and control measures. The information pertaining to description, host range, vectors, etc., will undoubtedly prove to be of value to research workers, teachers and students. It is interesting to find that *Ruga gossypii* Holmes passes to *Malvaviscus arborens* Cav. in Africa in which it produces leaf curl. However, in India, the commonly occurring leaf curl of *M. arborens* does not pass to cotton, (Mukherjee, A. K. and S. P. Raychaudhuri, *Indian J. Hort.* 21 : 176-177, (1964) and the cotton leaf curl is not known to occur in India. At the end, detailed references and symptom pictures of different diseases have been presented. Dr. Tarr has evidently taken considerable pains in reviewing and pooling the information in a precise manner.

S. P. RAYCHAUDHURI

———

Die Pilze (Grundzüge ihrer Entwicklungsgeschichte und Morphologie) By E. Gäumann ; 541 pp., 610 figs., 2nd Edition (1964).

Price : 66 Swiss Francs ; Publisher : Birkhäuser Verlag, Basel and Stuttgart.

The text under review is the second and enlarged edition of *Die Pilze* first published in 1949. An English version prepared by F. L. Wynd was printed in 1952. The book has come out of the press most regrettably a few months after the sad demise of Prof. Gäumann.

In the Introduction, Prof. Gäumann defines Fungi or *Mycetes* as chlorphyllfree Thallophyta exclusive of Bacteria and Myxomycetes. From the Bacteria they differ in the possession of true cell nuclei which are provided with chromosomes and nucleoli ; they are different from Myxomycetes because their cell walls contain cellulose and/or chitin. In addition to Myxomycetes organisms of uncertain relationship such as Acrasiales, Labrinthulales, Hydromyxales, Trichomycetes, etc., have been excluded from consideration in the text.

Prof. Gäumann has retained the class Archimycetes as distinct from the other so-called Lower Fungi. In the class Phycomycetes

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

6 orders have been recognized instead of 5 as was the case in the older text. The Order added is Hypochytriales which was perhaps earlier considered by Prof. Gäumann as nothing more than a family of Chytridiales.

It is in the class Ascomycetes that major reshuffles have been effected and it does indicate the ferment which the classification of this group is undergoing. The wall characters of the ascus as also the morphology of the ascus apex have been accorded primary importance. Accordingly, the class Ascomycetes has been divided into 2 sub-classes, Prototunicatae and Eutunicatae. The former embraces those fungi in which the mature ascospores are released after degeneration or gelatinisation of the ascus wall. This sub-class includes 9 Orders. Orders such as Endomycetales, Protomycetales and Taphrinales which have been traditionally accepted as belonging to Hemiascomycetidae have not separated in the new classification outlined in the text. The Taphrinales have been placed the group Unitunicatae of sub-class Eutunicatae while Endomycetales and Protomycetales have been classified under Prototunicatae.

The sub-class Eutunicatae has been divided in two groups, the Unitunicatae and Bitunicatae. The latter includes the stromatic Ascomycetes containing the three Orders Myriangiales, Dothiorales, and Pseudosphaeriales. All the rest of the Ascomycetes constitute the group Unitunicatae which contains 10 Orders.

It is a treat to go through the illustrations of Ascomycetous fungi in this new edition. Especially noteworthy are the drawings of Emil Müller and von Arx. Illustrations of the various patterns of ascus tip morphology unearthed in recent years are also quite illuminating.

The Basidiomycetes have been divided into two sub-classes, the Holobasidiomycetes and Phragmobasidiomycetes. The former has been classified into 5 Orders : Aphyllophorales, Agaricales, Agricogastrales, Gastrales and Phallales. The inclusion of information and photographs on genera such as *Phlebomycena*, *Poromycena* and *Ixechinus* having a novel net-like hymenophore under Agaricales is especially worthy of mention.

In the sub-class Phragmobasidiomycetes, 4 Orders, Tremellales, Auriculariales, Uredinales, and Ustilaginales have been included. The treatment of the Order Uredinales is quite comprehensive and well-integrated. It may be noted that Prof. Gäumann had a special interest in this group. It has been divided into 6 families. The traditional division into 2 families—Melampsoraceae and Pucciniaceae—was never accepted by Prof. Gäumann. Recent work also indicates that there is a greater diversity of telial and teliospore development than was implied in the older classification.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Three modes of dikaryotisation (instead of five described by Buller) have been given : 1, through spermatial fusion ; 2, through "somatogamy" ; and 3, through Buller phenomenon. The basal cells in the Rust Fungi have been compared with the ascogonia of *Neurospora*. A well-illustrated account of the development of all the spore forms (pycnia, aecia, uredia, and telia) has been provided. Under the heading of 'Modifications in the Development of Rust Fungi' 5 types of life-cycle variations have been considered. These are : long-cycled rusts with repeating aecia, the brachy, the opsis, the micro and the endo types. What seems to be a most daring change is the transfer of the rust genus *Goplana* to the family Septobasidiaceae of Auriculariales.

Knowing as we do the views of Prof. Gäumann it is not unexpected that Fungi Imperfecti have been dismissed in a little more than one page.

In the Bibliography the literature coverage is adequate but the omission of titles of original papers is a deficiency which could have been eliminated in the new edition.

The English edition of 1952 was one of the most frequently consulted texts in the Mycology classes and the same would be the case with this new edition too, if only it finds a rapid translation into English. The reviewer hopes someone somewhere has already undertaken this task though he is not aware of it.

M. M. PAYAK

Allgemeine Viruspathologie der Pflanzen by Von Dr. Erich Köhler, 178 pp. 86 illustrations, 1964, DM. 48/-). *Paul Parey, Berlin 61.*

Dr. Köhler should be congratulated for bringing out this very nice handy volume which deals with almost all aspects of plants viruses in a very condensed form. It has nine chapters viz., effect of infection on host plants ; morphology and Physico-chemistry of virus types ; infection and illness ; transmission ; the virus types, its differences and classification ; differences within the types, variability and mutability ; mixed infec ions and virus interference ; resistance and specialization ; epidemiology and ecology. There is an excellent list of literature after different chapter, which is up-to-date. The form and size of virus particles, the structure of the virus particles as well as other properties of the viruses have been very well described under three sub-sections in chapter 2. In the chapter on mixed infection on virus interference, the inferences of different variants of the same virus type, interference of different types of viruses as well as interferences in insect vectors have been very well dealt with.

The publication will certainly be very useful for the teachers, students as well as research workers specially in the field of plant virology and it will be warmly welcomed by all the scientists in the field of virology.

S. P. RAYCHAUDHURI

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PHYTOPATHOLOGICAL NEWS

Virologist Yasuo Komuro, Chief of Laboratory of Plant Virus Classification, Institute of Plant Virus Research, Chiba (Japan) visited Division of Mycology, Indian Agricultural Research Institute, New Delhi, and gave a talk on 'Some important plant virus diseases in Japan', on July 24, 1965.

Indian Phytopathological Society congratulates Phytopathologist K. Ramakrishnan on his assumption of the office of Associate Dean, College of Agriculture and Research Institute, Coimbatore (Madras).

Phytopathologist S. Sinha, Head of Department of Botany, Agra College, Agra (U. P.) has returned to India after a 3-month visit to Denmark and Holland.

Retirement : R. N. Tandon, Professor and Head of the Department of Botany, Allahabad University, has retired after a long and meritorius service.

Announcements

Annual General Meeting of the Indian Phytopathological Society, will be held on January 1 and 2, 1966 at Chandigarh (Punjab). This will include a business meeting, 3 symposia, Mundkur Memorial Lecture and a popular lecture.

Six-day International Symposium on 'Recent Advances in Diseases of Tropical Plants' will be held under the auspices of I. P. S., starting from December 27, 1966. Many eminent Pathologists from different parts of the world are expected to attend.

A symposium on 'Recent advances in the development, production and utilization of medicinal and aromatic plants in India' under the auspices of Central Indian Medicinal Plant Organisation (CIMPO) will be held at Lucknow on 12th, 13th and 14th January, 1966.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

PAGES

Phytopathological Notes

Serological relationship of sunnhemp mosaic virus with some other mosaic viruses commonly occuring in Delhi—*G. P. S. Anand and H. S. Sahambi* 204

Preliminary biochemical observations of leaf spot disease of groundnut—*V. Ravindra Nath, L. G. Kulkarni and B. Appaji Rao* 206

Some important observations on the staining of bacterial flagella with modified Bailey's method—*Y. P. Rao and V. K. Mathur* 208

Notes on powdery mildews from India-III.—*G. Lall and J. N. Gupta* 210

Natural occurrence of specialised forms of Puccinia graminis and P. striiformis on Lolium perenne.—*D. P. Misra, S. T. Ahmad and Sheodhan Singh* 214

Relative efficacy of fungicides for the control of Cladosporium variabile causing leaf spot disease of Spinach-(*Spinacia oleracea*)—*R. L. Mathur and S. P. Sehgal* 215

A Fusarium sp. with cellulolytic and lignolytic properties from manure pit—*G. Rangaswami and P. Rajasekaran* ... 217

Anthracnose of Celosia argentea var. cristata in Delhi—*R. L. Munjal and J. N. Gupta* 218

Occurrence of Tristeza virus in citrus in Northern India—*T. K. Nariani, H. S. Sahambi and B. L. Chona* ... 220

Reviews 221

Phytopathological News 224

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1965

President	...	... M. J. THIRUMALACHAR
Immediate Past President	...	... S. SINHA
Vice-President	...	... K. S. THIND
Secretary-Treasurer	...	... S. P. RAYCHAUDHURI (1965-67)
Joint Secretary	...	... M. M. PAYAN (1965-67)

COUNCILLORS

Northern Zone	...	... T. K. NARIANI
Mid-Eastern Zone	...	... B. M. GUPTA
Eastern Zone	...	... S. N. BANNERJEE
Central Zone	...	... B. L. MATHUR
Western Zone	...	... S. P. CAPOOR
Southern Zone	...	... G. RANGASWAMI

EDITORIAL BOARD FOR 1965—67

R. PRASADA

Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU
L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

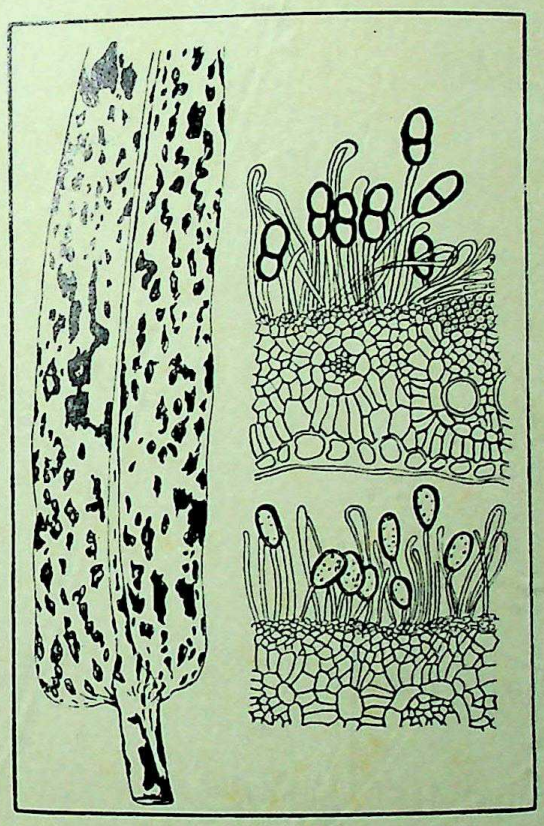
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVIII 1965 NUMBER 3



PUBLISHED FOR

THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Chemical treatment of seed and nursery bed to control damping-off of cabbage—*J. S. Grewal and R. P. Singh* ... 225

Performance of oat varieties against Puccinia coronata avenae and Puccinia graminis avenae—*D. P. Misra, Sheodhan Singh and S. T. Ahmad* 229

A mosaic disease of Tori (Luffa acutangula)—*D. K. Mitra and T. K. Nariani* 233

Hainesia lythri on Strawberry—A new record for India—*H. C. Phatak and M. M. Payak* 237

Collar-rot of Loquat by Sclerotium sp. and its control—*I. N. Tandon* 240

Assay of Bordeaux Mixture as soil fungicide—*O. P Sharma and A. C. Jain* 246

The effect of stem rust on the uptake and incorporation of P^{32} by wheat seedlings—*K. L. Mukherjee and Michael Shaw* 250

Mutation in Colletotrichum falcatum, the causal organism of red rot of sugarcane-VI. Induced biochemical mutants as test organisms for bioassay of P-aminobenzoic acid and Biotin—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* 253

A pathogenic Cunninghamella on pumpkin and its control—*Rajendra K. Grover* 257

Studies on Puccinia hordei I. Physiologic specialization and screening of varieties—*L. M. Joshi, R. Prasada and L. B. Goel* 267

Effect of hexose phosphates and ultraviolet irradiation on perithecial development in Glomerella tucumanensis—*A. K. Dutta, B. L. Chona and B. S. Bajaj* 274

(Contd on. 3rd cover)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHEMICAL TREATMENT OF SEED AND NURSERY BED TO CONTROL DAMPING-OFF OF CABBAGE

J. S. GREWAL AND R. P. SINGH

(Accepted for publication May 6, 1965)

Damping-off causes heavy losses to cabbage seedlings at vegetable breeding sub-station, Katrain, and other places wherever cabbage nursery is grown. In view of the heavy losses caused by damping - off pathogens to cabbage in nussery beds, an attempt was made to find out an effective chemical treatment for the control of this disease. Three experiments were conducted to test the relative efficacy of some proprietary fungicides against damping-off by seed and soil-drench treatments separately as well as together. The results obtained are reported herein.

EXPERIMENTAL : Isolations made from damped-off seedlings revealed the presence of *Pythium aphanidermatum*, *Rhizoctonia solani*, and *Rhizoctonia bataticola*. Cultures were purified by single hyphal tip method and pure cultures were used for further experimentation. All experiments were conducted with four replications in 4″ pots in unsterlized soil, inoculated with above mentioned three fungi. They were multiplied and added to the soil as described by Govindappa and Grewal (1965)[1]. Seventy-five seeds of cabbage (variety Golden Acre) were sown in each pot and proper controls were maintained in all experiments. Pre - and post-emergence damping-off were calculated on the basis of seeds germinated in uninoculated control and total emergence recorded 5 days after sowing, respectively.

Seed treatment : Cabbage seeds were treated with six fungicides, viz., Captan [N-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide], Arasan [bis (dimethylthiocarbamoyl) disulphide], Brassicol (pentachloronitrobenzene), Agrosan GN (tolylmercury acetate) and Copper carbonate for 30 minutes on a mechanical shaker to give uniform coating of fungicide on seeds. The fungicides used and the data obtained regarding pre - and post-emergence damping-off are summarized in Table 1.

It would be clear from the data in Table 1, that highest germination was recorded in Arasan treated seeds which was closely followed by Captan. Seeds treated with Captan and Arasan showed 4.4 and 1.3 per cent pre-emergence, 6.9 and 11.7 per cent post-emergence damping-off respectively, as against 48.8 per cent and 47.8 per cent in the untreated control. Copper carbonate proved phytotoxic. It reduced germination and also exerted phytotoxic effect on the germinated seedlings. The final stand after damping-off in Copper Carbonate, was 29 as against 60 in the untreated control, 200 in Captan and 198 in Arasan. Evidently, the seed treatment with Captan or Arasan is quite effective against damping-off of cabbage seedlings.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Efficacy of different fungicides against damping-off of cabbage

Fungicide	Dosage in per cent	Total emergence	Percentage damping-off		Final stand
			Pre-emergence	Post-emergence	
Captan	0.2	215	4.4	6.9	200
Arasan	0.2	222	1.3	11.7	198
Phaltan	0.2	210	6.7	25.2	157
Brassicol	0.2	174	22.7	33.3	116
Agrosan GN	0.2	149	37.8	34.9	97
Copper carbonate	0.2	83	63.1	65.0	29
Control (inoculated)	—	115	48.8	47.8	60
Control (uninoculated)	—	225	—	—	225

TABLE 2 : Efficacy of different fungicides against post-emergence damping-off of cabbage seedlings

Fungicide	Active ingredient	Dosage in per cent	Percentage post-emergence damping-off
Parzate dry	(zinc ethylenebis dithiocarbamate)	0.2	30.0
Captan	[n-(trichloromethylthio)-4-cyclohexene-1-2-dicarboximide]	0.2	30.9
Fytolan	(copper oxychloride)	0.2	35.7
Panogen	[cyano(methylmercuri) quanidine]	0.05	38.3
Arasan	[bis (dimethyl thiocarbamoyl) disulphide]	0.2	43.3
Rhizoctol	(methyl arsinic sulphide)	0.05	83.7
Control (drenched with water)	—	—	85.7

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Soil-drench treatment : The untreated seeds were sown in pots and soil in the pots was drenched with six fungicides 5 days after sowing. This was followed by second soil-drench at an interval of 7 days. Fifty ml. of fungicide suspension was used to drench each pot. The data regarding post-emergence damping-off were recorded twice a week (dead seedlings were removed after each counting) till the seedlings were two weeks old. The inoculum being heavy, the incidence of post-emergence damping-off was quite high. The fungicides and their dosages used as well as final data regarding post-emergence damping-off are given in Table 2.

The data in Table 2 indicate that soil-drench treatment with 0.2 per cent suspension of Parzate dry and Captan in water reduced the incidence of post-emergence damping-off to 30.0 and 30.9 per cent, respectively, as against 85.7 per cent in the Control.

Combined seed and soil-drench treatments : An experiment was conducted to test the combined efficacy of two seed protectants and two soil-drench treatments. Seeds were treated with Captan and Arasan at the rate of 2 gm./Kg. of seed, selected on the basis of their efficacy against pre-emergence damping-off as reported in Table 1 and sown in pots. Four pots of each treatment were drenched twice at an interval of one week with 0.2 per cent suspension of Captan and Parzate dry respectively selected on the basis of results presented in Table 2. The first soil-drench treatment was given 3 days after sowing. The data obtained are summarized in Table 3.

TABLE 3 : Efficacy of combined fungicidal seed and soil-drench treatments against damping-off of cabbage

Seed treated with	Soil-drench with	Percentage damping-off	
		Pre-emergence	Post-emergence
Captan	Captan	5.3	0.9
Captan	Parzate dry	22.2	1.2
Captan	Water	22.2	14.9
Arasan	Captan	17.8	2.1
Arasan	Parzate dry	20.5	8.9
Arasan	Water	39.2	16.1
Control (Untreated)	Water	41.9	26.1

It would be observed that the combined seed and soil-drench treatments proved superior to either seed or soil-drench treatment alone in checking the damping-off of cabbage. Seed treated with Captan (0.2 per cent) and soil-drench with the same fungicide reduced

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the pre - and post-emergence damping-off to 5.3 and 0.9 per cent as against 41.9 and 26.1 per cent in the control respectively. Captan was followed by seed treatment with Arasan and soil-drench with Captan, where incidence of pre - and post-emergence damping-off was observed to be 17.8 and 2.1 per cent respectively.

Harrison (1953)[2] recommended Captan for the control of damping-off of tomato. Jack (1954)[3] got similar results with Flit-406 against damping-off of pea.

Present investigations have clearly shown that damping-off of cabbage can be effectively controlled by seed treatment with Captan at the rate of 2 gm./Kg. of seed weight followed by soil-drench treatment with 0.2 per cent suspension of Captan twice at an interval of 3 and 10 days after sowing.

SUMMARY

Pythium aphanidermatum, *Rhizoctonia solani* and *Rhizoctonia bataticola* were found to be associated with damping off of cabbage. Comparative tests with six fungicides namely, Captan, Arasan, Phaltan, Brassicol, Agrosan GN and Copper carbonate, showed that seed treatment with Captan and Arasan reduced the incidence of pre-emergence damping off to 4.4 and 1.3 per cent respectively, as against 48.8 per cent in the untreated control. Tests with six other fungicides, namely Parzate dry, Panogen, Captan, Fytolan, Arasan and Rhizoctol used as soil-drench fungicides showed that 0.2 per cent suspension of Parzate dry and Captan in water reduced the incidence of post-emergence damping-off to 30.0 and 30.9 per cent respectively, as against 85.7 per cent in the Control. Combined effect of seed and soil-drench treatments showed that seed treated with Captan (0.2 per cent) and soil-drench with the same fungicide reduced the pre-and post-emergence damping-off to 5.3 and 0.9 per cent, respectively, as against 41.9 and 26.1 per cent in the untreated Control. Copper carbonate however proved phytotxic.

ACKNOWLEDGEMENTS : Our grateful thanks are due to Dr. B. L. Chona, Head of the Division, Mycology and Plant Pathology for encouragement, keen interest and providing necessary facilities of work and going through the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

---

[1]Govindappa, M. H. and J. S. Grewal. *Ind. Jour. Hort.*, **22** : (1965).
[2]Harrison, A. L. *Phytopathology*, **43** : 291 (1953).
[3]Jacks, H. *New Zealand Jour. Agri. Res.*, **2** : 306-311 (1954).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PERFORMANCE OF OAT VARIETIES AGAINST PUCCINIA CORONATA AVENAE AND PUCCINIA GRAMINIS AVENAE

D. P. MISRA, SHEODHAN SINGH AND S. T. AHMAD

(Accepted for publication May 6, 1965)

Oat is a minor fodder crop in India. Recently varieties like Kent, Overland, Curt, White Algerian have been introduced by the Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi and are being tried for the purposes of breakfast cereals and fodder. The performance of some of these have been observed to be considerably superior to the indigenous varieties in field. In many countries either or both the rusts viz., *Puccinia coronata avenae* (crown rust) and *P. graminis avenae* (black rust) have been known to cause considerable losses to oat crop. In India, black rust of oat has been found to be prevalent only in Nilgiri Hills and 4 races have been identified by Mehta (1940). From North India, Payak and Misra (1963) and Misra *et al.* (1964) have identified 3 races of crown rust. In view of the increasing importance now being given to the cultivation of oats in the country, it was considered necessary to have information on the performance of available oat varieties against the two rusts. This might enable the release of suitable varieties of oat as also to embark upon programme of breeding resistant varieties, if necessary. In the present paper, the results of 88 varieties (exotic and Indian) of oats, tested in the seedling stage against the rust material so far met within the country, are reported.

RESULTS : Oat varieties were tested with races 227, 231 and 240 of crown rust individually and with a mixture of races 3, 4, 6 and 7 of black rust. The races of black rust were isolated about three decades ago and the existing seed of differentials was not found reliable for determining the purity of these races and therefore, the races were used in mixture for testing.

Out of 88* varieties thus tested, the following 57 varieties were found to be susceptible to all the races of the two rusts :

N. P. 1, Hyb. 1, Hyb. 2, Hyb. 3, Hyb. 4, Junagarh Farm 1, Junagarh Farm 2, Kanpur local, Layallpur local, I-251-32, II-97-84, II-51-9, III-242-56, IV-B-16-17, X-27, XI-A-24-30, XI-A-325-16, XII-B-93-51, XIII-B-116-36, XIII-B-116-36G, XIII-B-153-2, XIV-76-55, XV-75-73, XV-75-73G, B. S. 1, B. S. 2, B. S. 4, P. F. 2, Reed 12, Reed 14,

*18 out of 88 varieties were tested against crown rust races by Misra *et al.* (1964) and here in these varieties have been tested with *P. g. avenae*. However, the results have been consolidated in this paper for convenience.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE : Comparative reactions of 31 oat varieties with races of crown rust and black rust of oat.

Oat Varieties	*Puccinia coronata avenae* Races 227	231	240	*P. g. avenae* Races 3, 4, 6 and 7 (Mix).
1. I-80-40	I—S	I—S	I	S
2. VII-265	I—S	I—S	I	S
3. VIII-578	R	R	R	S
4. Iowa-103	I—S	R—I	R	S
5. Iowa-670	S	I—R	R	R
6. Budha	I—S	R—I	R	S
7. Gopher	S	S	R	R
8. Kandula	I—S	I—S	S	R
9. Markton	S	S	I—R	R
10. Mulga	I—S	R	R	S
11. Norton	S	S	R	S
12. Reed 10	S	S	R	S
13. Algeria	I—S	R—I	R	S
14. Angnoise	I	R—I	R	S
15. Breenker	I—S	I	R	S
16. Fulgum	S	I—S	R	S
17. Gidque	S	S	R	S
18. Overland	R	R	R	S
19. White Algerian	S	S	I	S
20. X-27	I	I	I	S
21. New Australian	I	R	I	S
22. Curt	S	S	S	R
23. Bondvick	R	R	R	SR
24. Ukrain	S	S	R	S
25. Landhafer	R	R	R	S
26. Bond	R	R	R	S
27. Saia	S	R	R	R
28. Appler	S	R	R	S
29. Santa Fe	I	R	R	SR
30. Trispernia	R	R	R	SR
31. Victoria.	R	R	R	S

N. B.—S—Susceptible ; I or SR—Intermediate or mixed type ; R—Resistant.

Sr. No. 1 to 17—seed received from Botanical Sub-Station, Indian Agricultural Research Institute, Pusa.

Sr. No. 18 to 22—seed received from Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi.

Sr. No. 23 to 31—Differentials of crown rust of oat.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Reed 19, Reed 19A, Reed 21, Reed 22, Reed 23, Reed 29, Iowa 105, Abundance, Advocated, Carston, Knota, Laggoon, Neb. 21, Palestine, S. Potato (seed received from Botanical sub-station, Indian Agricultural Research Institute, Pusa) ; Boris opus, Kent, Adliker, Dale, Ballidue Avon, Fleming Gold (seed received from Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi) ; Richland, Jonette, Minrus, Victory, Anthony.

Remaining 31 varieties showed different reactions to the material of rusts and their comparative reactions are set out in the table.

It is seen from the table that no single variety is resistant to all the races of the two rusts. Varieties VIII-578, Overland, Bondwick, Landhafer, Bond, Trispernia, and Victoria are resistant to crown rust races and Iowa 670, Gopher, Kandula, Markton, Curt, Saia, are resistant to black rust of oat.

CONCLUSION : In India, limited studies have been undertaken so far for determining the prevalence and distribution of races of crown rust and black rust of oat, and therefore, more races are likely to be found on comprehensive study. However, with the known races, 88 varieties of oat were tested and most of them have been found to be susceptible to races of both the rusts and remaining varieties were resistant to one or more races.

Bondwick, Trispernia, Landhafer, Victoria and Bond have been found to be resistant to the three races of crown rust and these varieties are already included in the set of differentials for crown rust races. As long as these varieties remain resistant to the new races that are picked up in the country, their derivatives are likely to be of promise against this rust. Landhafer and Victoria in U. S. A. are found to carry one gene in each for resistance to crown rust and Bond with two complementary factors for resistance (Litzenberger, 1949). These varieties are known to be good parents as donors for disease resistance but are not commonly cultivated (Stanton, 1955). However, Overland which has also been found resistant to the 3 Indian races of crown rust (227, 231, 240), is being cultivated in the states of Idaho, Montana, Oregon, Utah and Washington in U. S. A. (Stanton, 1955). Therefore, in North India where black rust of oat is not reported to occur, it may be worth while testing the suitability of Overland for cultivation.

Gopher, Markton, Iowa 670, Kandula, Curt and Saia have been found to be resistant to black rust of oat. Out of these, Gopher and Markton are under cultivation in a number of states in U. S. A. (Stanton, 1955). Therefore, in the penninsular India, where black rust is common and crown rust is not reported, these varieties may be taken for extensive yield trials. However, all the aforesaid six varieties may be of utility as donors for black rust resistance.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his guidance during

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the course of these studies. Thanks are also due to Dr. R. Prasada, Mycologist for his keen interest in the work and to Heads of the Divisions of Plant Introduction and Botany, Indian Agricultural Research Institute, New Delhi, for supplying the seeds of oat varieties.

Indian Agricultural Research Institute,
Plant Pathological Research Sub-Station,
Flowerdale, Simla-East.

REFERENCES

LITZENBERGER, S. C. (1949) Inheritance of resistance to specific races of crown and stem rust, to *Helminthosporium* blight and of certain agronomic characters of oats. *Iowa Agric. Exp. Sta. Res. Bull.* **370** : 454-96.

MEHTA, K. C. (1940) Further studies on cereal rusts in India. *Scien. Mongr.* **14** : Indian Council of Agricultural Research, 1-224.

MISRA, D. P., S. K. SHARMA, P. C. JOSHI AND SHEODHAN SINGH. (1964) Further studies on the crown rust of oat in India. *Indian Phytopath.* **17** : 138-141.

PAYAK, M. M. AND D. P. MISRA (1963) Physiologic specialisation in *Puccinia coronata* Corda, in India. *Indian Phytopath.* **16** : 15-20.

STANTON, T. R. (1955) Oat identification and classification. *U. S. D. A., Tech. Bull.* **1100** : 1-206.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A MOSAIC DISEASE OF TORI (LUFFA ACUTANGULA)*

D. K. MITRA AND T. K. NARIANI

(Accepted for publication May 29, 1965)

Luffa acutangula Roxb. commonly known as ridge gourd or *Kali tori* is cultivated throughout India as a popular vegetable. For the past several years the crop has been found to be affected with a mosaic disease suspected to be of virus origin. A brief account of the investigations conducted on the disease with a view to identify the causal virus is presented in this paper.

Symptoms : In the field, *Luffa* plants show a distinct mosaic mottling consisting of dark green and light green patches which are very conspicuous from a distance (Fig. 1). The dark green areas are slightly sunken giving the leaf lamina a wrinkled appearance. Accompanying these symptoms there is a gradual downward curling of the leaf margins. In older leaves the chlorotic areas increase, become circular and turn yellow. Affected leaves are reduced in size, thick and leathery. The infected plants are stunted in growth and bear fewer flowers and fruits.

Transmission : The disease was successfully transmitted by mechanical inoculation by rubbing the juice of infected leaves on healthy seedlings of *tori* using carborundum powder as an abrasive. The first visible symptoms appeared as early as on 4th day of inoculation in the form of yellowish pin head like dots or circular patches followed by distinct mosaic mottling. The disease, however, could not be transmitted by the aphids, *Myzus persicae* Sulz., *Aphis craccivora* Koch., *Rhopalosiphum maidis* Fitch or the red pumpkin beetle, *Riphidopalpa foveicollis* Lucas. About 1000 seedlings of *tori* raised in the Insect-proof glasshouse from seeds collected from fruits of mosaic affected plants in the field, remained healthy, indicating thereby that the virus is not transmitted through seed.

Physical properties : The virus in the standard extract** was found to be infective when heated to 55°C for 10 minutes but was rendered innoccuous when heated to 60°C for the same period. The crude sap diluted to 1 : 500 with distilled water was infective but was rendered non-infective when diluted to 1 : 1000. The longevity of the virus *in vitro* was found to be 4 – 6, 6 – 8 and 24 – 48 hours when the infective juice was stored at room temperature (38 – 41°C), 8 – 10° and 0°C respectively. The virus was found to be viable in the desiccated mosaic affected leaves of *tori* for 3 days but was inactivated after 4 days.

*Based on thesis submitted by the Senior author for M. Sc. degree of Indian Agricultural Research Institute, New Delhi.

**Standard extract was prepared by crushing the diseased leaves in a pestle and mortar adding distilled water at the rate of 1 ml. for each gram of leaf material.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Host range : Fifty six plant species belonging to thirteen families raised from seeds in the insect-proof glasshouse were tested by mechanical sap inoculation. The plants which did not show any symptoms were indexed by back inoculation to *Luffa acutangula* to determine if they were symptomless carriers. It was observed that the host range of the virus is restricted to Cucurbitaceae only. Successful transmission of the virus was obtained in *Luffa cylindrica* Roem, *Lagenaria siceraria* Standl. (Fig. 2), *Cucurbita moschata* Duchesne, *C. pepo* L. (Fig. 3), *Citrullus vulgaris* Schrad var *fistulosus*, *Momordica charantia* L. and *Trichosanthes anguina* L. All these plant species reacted with systemic mosaic symptoms. The following plant species were found to be insusceptible.

Nicotiana tabacum L. vars. Harrison special and White Burley, *N. glutinosa* L., *N. rustica* L., *Lycopersicon esculentum* Mill., *Capsicum annuum* L., *Datura stramonium* L., *Solanum melongena* L., *Cucumis sativus* L., *C. melo* L., *C. melo*. Var. *Utilissima*, *Cucurbita maxima* Duchesne, *Citrullus vulgaris* Schrad., *Phaseolus aureus* L., *P. mungo* L., *Vicia faba* L., *Glycine max* (L) Merrill., *Crotalaria juncea* L., *Cicer arieitnum* L., *Arachis hypogaea* L., *Cajanus cajan* (L) Millsp., *Dolichos lablab* L., *Lathyrus odoratus* L., *Pisum sativum* L., *Cyamopsis tetragonoloba* (L) Taub., *Tephrosia spectabilis*, *Lactuca sativa* L., *Chrysanthemum indicum* L., *Helianthus annus* L., *Amaranthus caudatus* L, *A. cruentus* L., *A. gangeticus* L., *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste and Reyn., *C. album* L., *Spinacea oleracea* L., *Brassica oleracea* L. var. *capitata* L., *B. oleracea* var. *botrytis* L., *B. caulorapa* Pasaq., *B. campestris* L. var. Sarson, *Abelmoschus esculentus* (L.) Moench, *Althea rosea* L., *Gossypium herbaceum* L., *Vitis vinifera* L., *Carica papaya* L., *Sesamum indicum* L., *Rosa sinensis* L. and *Zea mays* L.

Serological tests : The virus under study although distinct from *Cucumis* virus 1 due to its host range restricted to Cucurbitaceae, resembles *Cucumis* virus 2 in this respect. In order to determine whether these two viruses are serologically related, precipitin ring tests were conducted using the centrifuged leaf extract of the virus and an antiserum of *Cucumis* virus 2 C (Bottle gourd mosaic virus). The centrifuged leaf extract of *Cucumis* virus 2 C was also used with its homologous antiserum and normal serum to serve as controls. No ring formation was observed with the clarified sap of *tori* mosaic virus although a clear whitish ring was visible in case of *Cucumis* virus 2 C. This indicated that the *Luffa* mosaic and bottle gourd mosaic viruses are serologically unrelated.

DISCUSSION : The viruses causing mosaic symptoms on *Luffa* spp. which have been worked out in detail with regard to mode of transmission, host range and physical properties, are the cucumber mosaic virus (Doolittle, 1920 ; Nariani and Nyako, 1963), tobacco ring spot virus (Wingard, 1928), (*Cucumis* virus 2B and 2C causing mosaic in bottlegourd (Capoor and Varma, 1948 ; Vasudeva *et al.* 1949), watermelon mosaic virus (Anderson, 1954), muskmelon mosaic virus (Anderson, 1951), papaya mosaic virus (Capoor and Varma, 1958) and *Cucumis* virus 3 causing mosaic of vegetable marrow (Reddy and Nariani, 1963).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

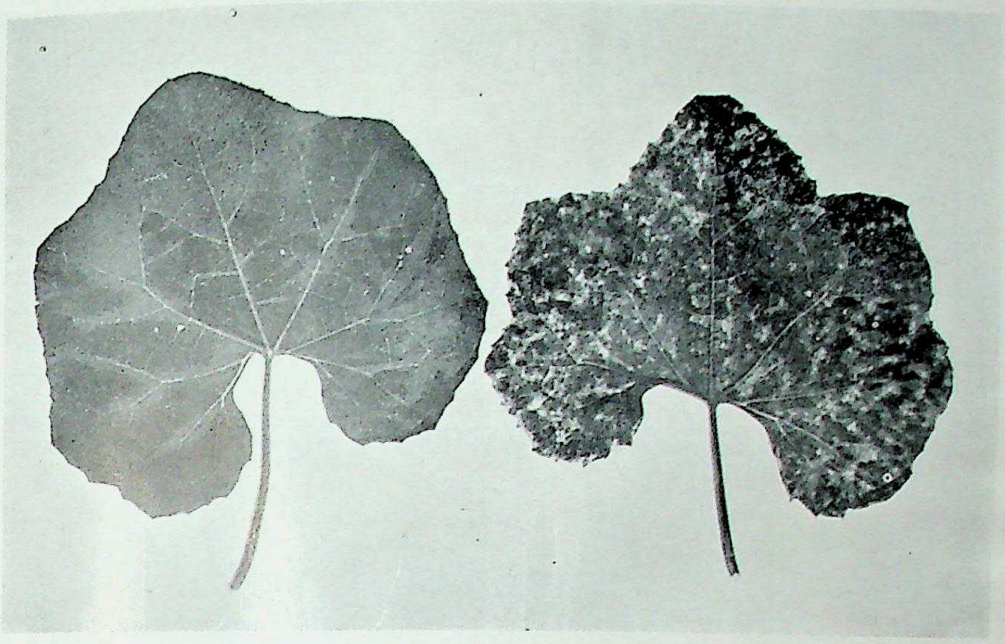


Fig. 1. Healthy and mosaic affected leaves of *Luffa acutangula* Roxb.

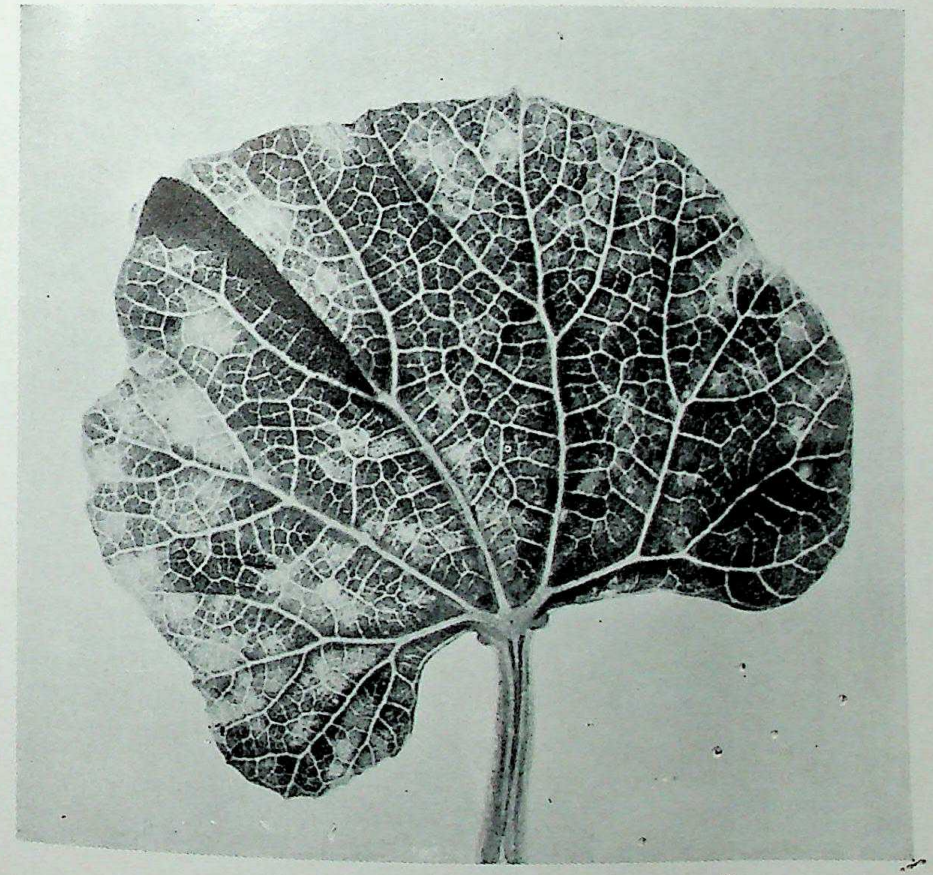


Fig. 2. Symptoms of *tori* mosaic virus on bottlegourd (*Lagenaria siceraria* Standl.).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

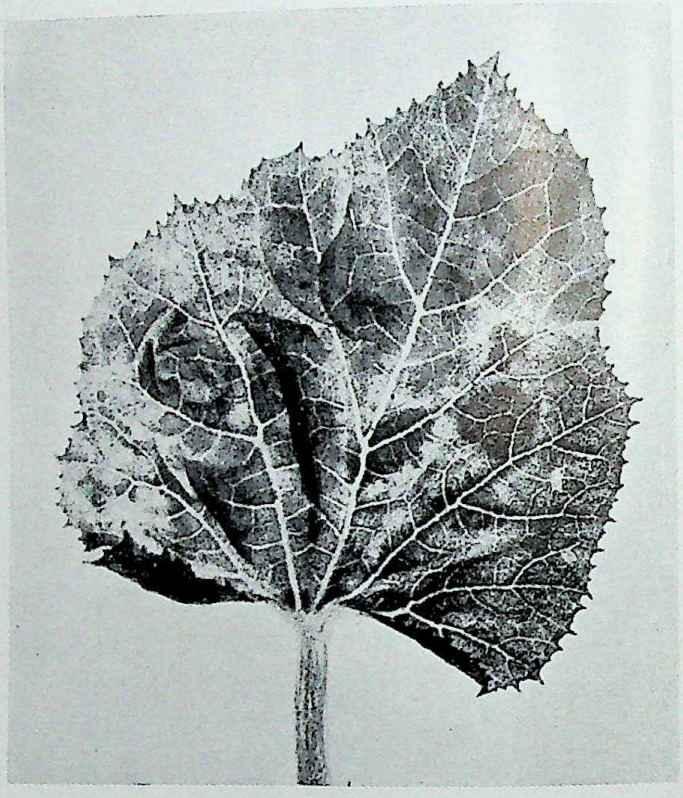


Fig. 3. Leaf of *Cucurbita pepo* L. showing mosaic and blistering when inoculated with *tori* mosaic virus.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The cucumber mosaic and tobacco ring spot viruses have a very wide host range covering several families and are, therefore, distinct from the *tori* mosaic virus. The papaya mosaic, watermelon mosaic and musk melon mosaic viruses differ from the virus under study in the physical properties and host range. Although *Cucumis* viruses 2B and 2C resemble the *tori* mosaic virus in having their host range restricted to Cucurbitaceae, they differ considerably in physical properties. Also *tori* mosaic virus did not react with the antiserum of *Cucumis* virus 2C in serological tests. The *tori* mosaic virus, however, resembles closely the *Cucumis* virus 3 causing filiformy of leaves in vegetable marrow (Reddy and Nariani, 1963), which was earlier reported by Vasudeva and Lal, 1943) on bottlegourd and has a thermal death point of 55–60°C, a dilution end-point of 1 : 500 and longevity *in vitro* of 6 hours and is transmissible to Cucurbitaceae only. Thus *Luffa acutangula* is an unrecorded host of the *Cucumis* virus 3.

SUMMARY

A virus causing mosaic of tori (*Luffa acutangula* Roxb.) commonly Cccurring at Delhi has been studied with regard to symptomatology, mode of transmission, host range and physical properties.

The virus was found to be sap transmissible by mechanical sap inoculation using carborundum powder as an abrasive, but was not transmitted through seed nor by *Myzus persicae* Sulz., *Aphis craccivora* Koch., *Rhopalosiphum maidis* Fitch or *Riphidopalpa foveicollis* Lucas. The host range of the virus was found to be restricted to familiy Cucurbitaceae. The virus was transmitted to *Luffa cylindrica* Roem, *Lagenaria siceraria* Standl. *Cucurbita moschata* Duchesne, *C. pepo* L., *Citrullus vulgaris* var. *fustulosus*, *Momordica charantia* L. and *Trichosanthes anguina* L., producing systemic mosaic symptoms.

The causal virus was found to have a thermal death point of 55–60°C, dilution end point between 1 : 500 and 1 : 1000 and longevity *in vitro* of 4-6 hours at room temperature (38-41°C), 6-8 hours at 8-10°C and 24-48 hours at 0°C. It was viable in the desiccated mosaic affected leaves for 3 days but was inactivated after 4 days.

The causal virus was serologically unrelated to *Cucumis* virus 2C and was identified as *Cucumis* Virus 3.

ACKNOWLEDGEMENTS : Grateful thanks are due to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology for his keen interest and encouragement and for providing necessary facilities for work as also to Shri G. P. Anand for supplying the antiserum of the *Cucumis* virus 2C.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

ANDERSON, C. W. (1954) Two watermelon mosaic strains from Central Florida. *Phytopathology* **44** : 198-202.

————————(1954) Two muskmelon mosaic strains from Central Florida. *Phytopathology* **44** : 371-374.

CAPOOR, S. P. AND P. M. VARMA (1948) A mosaic disease of *Lagenaria vulgaris* Ser. in Bombay province. *Curr. Sci.* **17** : 274-375.

———————— AND ————————(1958) A mosaic disease of papaya in Bombay. *Indian J. agaric. Sci.* **28** : 225-234.

DOOLITTLE, S. P. (1920) The mosaic disease of Cucurbits. *U. S. Dept. Agr. Bull.* 879.

NARIANI, T. K. AND K. O. NYAKO (1963) A mosaic disease of tobacco caused by *Cucumis* virus 1. *Indian Phytopath.* **16** : 313-316.

REDDY, K. R. C. AND T. K. NARIANI (1963) A mosaic disease of tobacco caused by *Cucumis* virus 1. *Indian Phytopath*, **16** : 260-267.

VASUDEVA, R. S. AND T. B. LAL (1943) Mosaic disease of bottlegourd. *Indian J. agric. Sci.* **13** : 182-192.

VASUDEVA, R. S., S. P. RAYCHAUDHURI AND J. SINGH, (1949) A new strain of *Cucumis* virus 2. *Indian Phytopath.* **2** : 181-185.

WINGARD, S. A (1928) Hosts and symptoms of ringspot—a virus disease of plants. *J. Agri. Res.* **23** : 127-153.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

HAINESIA LYTHRI ON STRAWBERRY - A NEW RECORD FOR INDIA

H. C. PHATAK AND M. M. PAYAK

(Accepted for publication May 29, 1965)

During July, 1964, a severe spotting on strawberry (*Fragaria* × spp.) leaves at Delhi was observed. Initially, the infection was seen as small ferrugineous, roundish to oval spots (of about 5 mm. diameter) with light centres. On enlargement, the spots covered large areas of the leaf lamina. The leaves, as a result, got blighted. On such diseased portions minute pycnidia of the fungus were borne. Infection was noted on the following exotic varieties grown for experimental purposes at Indian Agricultural Research Institute : *Albriton, Blakemore, Catskill, Cavalier, Gem, Howard-17, Hybrid-2, J. F. No. 2, Macheranch, Friihernt, Madam Moutot, Mastodan, N. H. Round Head, Premier, Queen of Australia, Rearguard, Red coat, Royal Round, Royal Sovereign, Stelemaster, VL 9* and an Indian variety - *Kulu* (*Sour*).

MATERIAL AND METHODS : The fungus was isolated from the diseased leaves of strawberry (var. *Royal Sovereign*). A spore suspension from a purified culture was used as an inoculum. Inoculations on the leaves and petioles of healthy plants (var. *Dilpasand*) were made in an insect proof glasshouse. The leaves of plants in one set of two pots were pin-pricked while in another set they were gently rubbed with carborundum powder. The purpose in both the cases was to cause slight injury. The leaves in two pots were directly smeared with spore suspension without causing any injury, while two pots were kept as control on which only sterile water was sprayed. The plants were incubated for 48 hours.

RESULTS : Infection appeared within 8-10 days in the 2 sets of 4 pots where the leaves had been slightly injured. The plants left as control and those on which spore suspension had been applied without injury remained free of infection. In the first set of 2 pots the symptoms *i.e.*, spotting developed around the pin pricks on the leaves while in carborundum-rubbed leaves it was irregular. The fungus was readily re-isolated from the infected leaves produced as a result of artificial inoculation.

MORPHOLOGY OF THE FUNGUS : The pycnidia are beaked and are the only fructifications found on the leaves. They are epiphyllous, sparse, scattered, subepidermal, innate, erumpent, carbonous, ostiolate, and have a long neck. They measure 120—250μ in height and 95—250μ in width. The neck is 60—125μ long and 60—110μ wide and has round to flattened or truncate apex (Figs. 2 and 3) in the centre of which the ostiole is located. The conidiophores (Figs. 4 and 5) are simple and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

unbranched or at the most branched only once. The conidia (Fig. 6) are cylindrical, acerose, occasionally curved, individually hyaline but in mass light amber to light pink, with round or slightly tapered ends, slightly narrowed in the middle, and 1–2 guttulate. They measure $4-8{\cdot}5 \times 1.2-1.6\mu$.

In culture, the fungus at first produces a mycelium which is dull white in colour. The hyphae are thin and hyaline. They spread aerially and cover the walls of the culture tube. The pycnidia are the first fructifications to appear within 3-6 weeks. They agree in all characters with those found on the host except that in culture they develop in clusters (becoming botryose) and are slightly smaller in size. Occasionally the clustering is very pronounced and they appear as thick, dark, sclerotic structures attaining a size of even 1 cm. The sporodochia (Fig. 1) arise later. They are flattened, discoid and dark but have lobed margins which are hyaline. These produce conidia in such large numbers that big, light amber-coloured cirrhi or spore-horns result which get curved and reflexed. This appears to be a characteristic and distinctive feature of the fungus in culture. The conidia morphologically are quite similar to those produced in the pycnidia and these are embedded in a gelatinous and sticky matrix.

IDENTITY : The perfect stage of the fungus which has not been found in India, has been identified as *Pezizella lythri* (Desm.) Shear and Dodge. It is now called as *Discohainesia oenotherae* (Cke. & Ell.) Nannf.

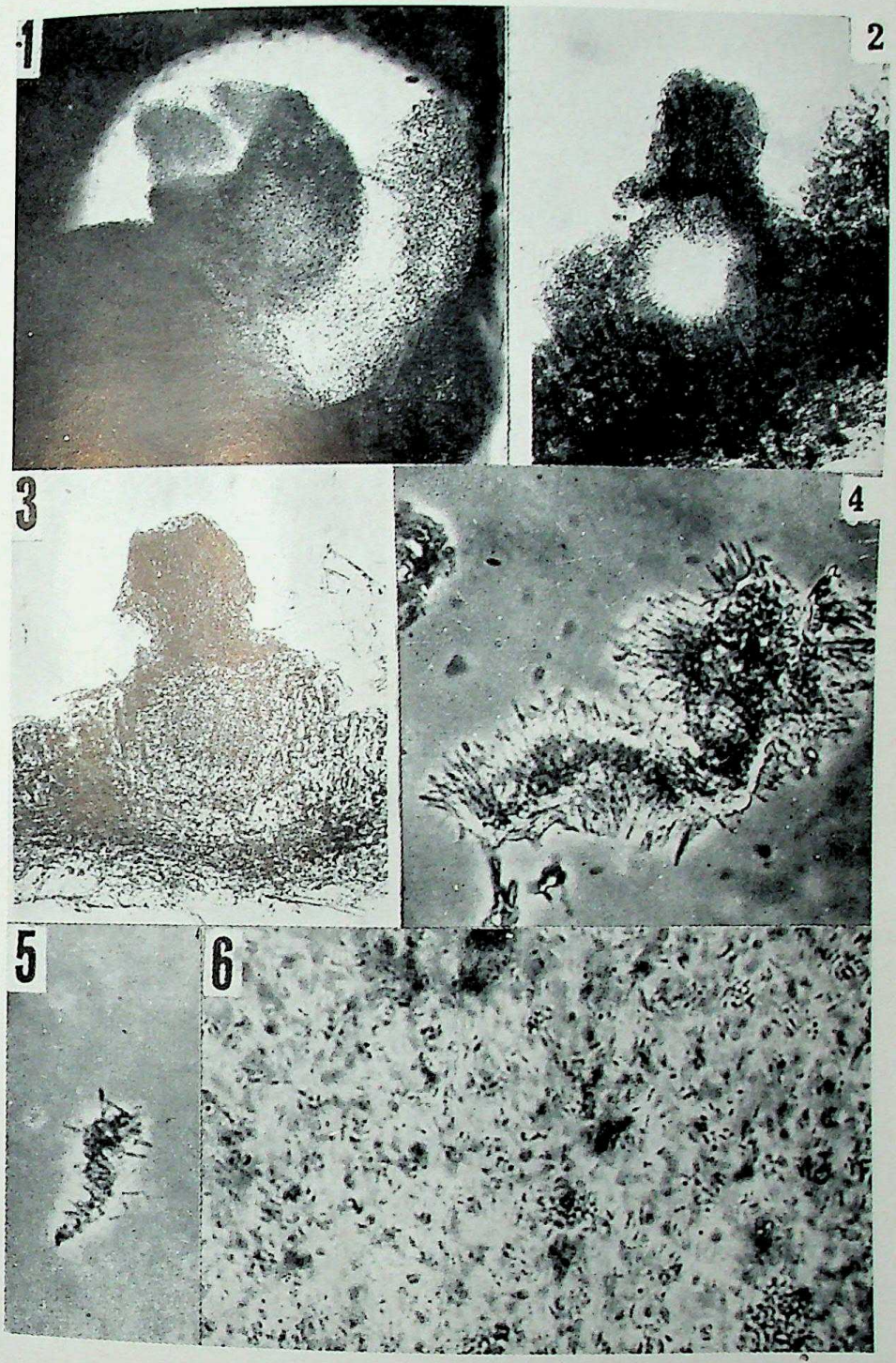
According to Shear and Dodge (1921), the pycnidial stage of this fungus as found on some plants other than strawberry is referrable to the genus *Sclerotiopsis*. They have described the pycnidia to be flattened with irregular splitting. In our material, however, the pycnidia have a long neck with flattened or round apex and are ostiolate. These have been found on strawberry leaves and their characters do not agree with those of the genus *Sclerotiopsis*. Moreover, according to Petrak (1951), this genus is a synonym of *Pilidium* Kunze. Grove (1937) found *H. rubi* Sacc. associated with, and parasitizing uredial pustules of *Phragmidium violaceum* developing on leaves of *Rubus rusticanus*. *Hainesia lythri* (Desm.) Höhn. *sensu stricto* has been considered by Grove as *Leptothyrium macrothecium* Fuckel. However, it seems preferable to accept the treatment and limits of this taxon as proposed by Shear and Dodge (1921) because of the considerable variability met within this fungus. As the fungus has also been found to produce sporodochia in culture, there is little doubt that it belongs to *Hainesia*. This is one of those genera of *Fungi Imperfecti* in which conidial stage is formed either in an acervulus, sporodochium or pycnidium.

The culture has been deposited in the Indian Type Culture Collection, at Indian Agricultural Research Institute, New Delhi.

DISCUSSION : The results of inoculation confirm the observation of Shear and Dodge (1921) that the fungus is a weak parasite and it incites disease only when it is able to gain entry through wounds, insect bites, or

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 1—6. 1. A flattened sporodochium from culture; note released conidial mass. × 100. 2 and 3. Transection of strawberry leaf showing pycnidia; note long neck, × 160. 4 and 5. Parts of pycnidium showing conidiophores and some attached conidia. ×480. 6. Part of the spore cirrhi from sporodochium in culture. × 480.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

injury caused by other agencies. The high incidence of the disease noted at Delhi during the rainy season of 1964 perhaps may have been due to the close proximity of the strawberry leaves to the ground making them more vulnerable to injury. It is possible that the soil or sand particles coming in contact with the leaves might be acting as abrasives. In the U.S. the fungus is known to incite tan rot of fruits and secondary rot of leaves and stolons. It has also been found associated with black root or root rot (Weiss and O' Brien, 1952 ; Demaree, 1948) of strawberry.

Summary

A fungus associated with leaf blight of strawberry at Delhi has been identified as *Hainesia lythri*. Inoculation work has shown that it is a weak pathogen and incites blight only when the leaves get injured. It has been suggested that its widespread occurrence in Delhi might be due to the injury that might be resulting from abrasive action of sand or soil particles on the leaves. In culture both, sporodochia and pycnidia have been obtained. The fungus characteristically produces amber coloured spore cirrhi. The pycnidia have a prominent neck and are ostiolate. Such types of pycnidia have not been reported earlier to be occurring in this fungus.

Acknowledgement : We wish to record our thanks to Drs. R. Prasada and S. P. Raychaudhuri for encouragement and facilities.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

References

Demaree, J. B. (1948) *Diseases of strawberries*. U. S. Dept. Agric. Farm. Bull. No. 1891.

Grove, W. B. (1937) *British stem and leaf-Fungi. (Coelomycetes)* **2**, 406 pp.

Petrak, F. (1951) Ergebnisse einer Revision der Grundtypen verschiedener Gattungen der Askomyzten und *Fungi imperfecti. Sydowia* **5** : 328-356.

Shear, C. L. and B. O. Dodge (1921) The life history and identity of *Patellina fragariae*, *Leptothyrium macrothecium* and *Peziza oenotherae*. *Mycologia* **13** : 135-170.

Weiss, F. and M. J. O'Brien (1953) *Index of plant diseases in the United States. Plant Dis. Reptr.* Special Publication No. 1, 1263 pp.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

COLLAR-ROT OF LOQUAT BY SCLEROTIUM SP. AND ITS CONTROL

I. N. TANDON

(Accepted for publication July 3, 1965)

The author[1] described a collar-rot and root rot of loquat (*Eriobotrya japonica Lindl.*) by *Diplodia natalensis* Pole-Evans. In an attempt to improve the procedure of artificial inoculation with this fungus, inoculations were carried out by inserting a piece of infected bark, obtained from a naturally infected tree, in between the bark and the wood of healthy trees. Although this method of inoculation did not reproduce the disease in any of the inoculated plants, except in one, where a white weft of fungal strands was observed on the surface of the bark which turned brown. The infection increased very rapidly and isolations carried out from the infected bark yielded a species of *Sclerotium*. This led the author to suspect that the fungus may be associated with the serious disease of collar-rot of loquat. Therefore, studies with this fungus were undertaken to find out how far it was responsible for producing the collar-rot phase of the disease and experiments to control the disease were also conducted.

MATERIAL AND METHODS : All the loquat plants selected for inoculation and control experiments were seedlings and their age varied from 4 to 7 years. For producing the disease artificially the region of collar or branch to be inoculated with the fungus was first disinfected by swabbing with alcohol and a two inch long cut made by means of a sterile knife. On both sides of the cut, a piece of pure fungus culture, grown on potato dextrose agar, was placed in between the bark and the wood. The collar or the branch at the inoculated region was then covered with moist cotton which was wrapped with an alkathene sheet to provide constant moisture.

For control experiments, loquat plants were first artificially inoculated with *Sclerotium* sp. in the last week of March, 1962. When about half the girth of the stem was infected, which took about 3 weeks, the infected bark alongwith approximately 1.5 cm. of the healthy bark all round was scraped off by means of a knife. The width in the centre of the scraped off area was measured at the time of scraping. The circumference of the collar at the place of inoculation as well as the width left uncovered by callus was measured in November, 1963.

Preparation of various pastes and solutions :

(a) Shell copper paste—prepared by mixing 1 lb. of Shell copper (copper oxychloride) and ½ lb. of slaked lime in ½ gallon of water.

[1]Tandon, I. N. (1960). *Hort. Adv.* 4 : 115-121.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(b) Bordeaux paste—prepared by dissolving 1 lb. of copper sulphate and $1\frac{1}{2}$ lb. of slaked lime, each in $\frac{1}{2}$ gallon of water separately and then mixed.

(c) Chevestelon solution—prepared by mixing a 6 per cent solution of copper sulphate to a 6 per cent solution of potassium bichromate, when the two solutions had cooled.

EXPERIMENTAL RESULTS : *Pathogenicity* : A large number of inoculations were carried out in the manner described already. Controls were maintained by inserting a piece of sterile potato dextrose agar medium in place of the fungus. The inoculated bark usually took the infection within three days and turned brown, whereas, the control did not take the infection. The browning increased rapidly and prominent white weft of fungal strands was often produced on the surface of the bark. After about two weeks, the characteristic brown, round or slightly irregular sclerotia or resting bodies of the fungus resembling radish seeds were often formed.

Gradually the entire collar or the inoculated branch turned brown all round, but the infection always did not penetrate deep into the bark. Whenever the bark was completely infected girdling the branch or the collar, it usually resulted in the death of the branch or plant after sometime. However, sometimes the entire branch or the collar was apparently girdled and even the leaves started turning yellow, but after sometime the plant recovered. A close observation revealed that the infected bark dried out and was delimited by callus formation along some of the healthy area. The healthy bark gradually developed and the infected bark was thrown off.

Artificial inoculations of the collar in different months revealed that most of the inoculations carried out between March and May resulted in the death of the plants whereas, those carried out in other months rarely resulted in the death of the plants though infection of the bark occured in varying degrees in all cases.

Infection of the bark occured even when no injury was made and the fungus was placed directly on the bark covered with moist cotton and alkathene. But, the incision method described above and followed throughout the investigations was more successful.

It may be mentioned that the discolouration caused by *Sclerotium* sp. was brownish and was lighter in colour than that caused by *Diplodia* sp. where the infected bark was dark brown to black in colour and often developed large number of pycnidia on its surface. In cases of inoculations with *Sclerotium* sp., the fungus could be reisolated from the infected region in the early stages till the inoculated collar or branch was almost completely girdled, but by the time the plant or the branch died, this fungus could rarely be reisolated and *Diplodia* sp. was invariably found to be associated with it.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Effect of temperature on the growth of Sclerotium sp. :

The growth of the organism was studied at different temperatures on potato-dextrose-agar. The maximum, minimum and optimum temperatures for its growth were found to be between 35–39°C, 12–14°C and 27–30°C respectively.

Sclerotia were formed in culture in about 7 to 14 days at room temperature (24–30°C) ; their formation being in abundance at the periphery of the petridish but some were scattered over the entire surface. A large number of these were formed along the line of scratch made on the culture by means of a sterilized needle. Their formation usually started as whitish mycelial knots which gradually became hard turning from light brown to dark resembling radish seed in size and appearance.

Control Experiments :

Experiments were carried out with *Sclerotium* sp. and the following treatments were given : (1) control (inoculated and allowed to develop), (2) scraping off of the infected bark without application of any paste, (3) scraping off of the infected bark and application of one of the following pastes to the scraped off area : (a) Bordeaux paste, (b) Shell copper paste and (c) Chevestelon solution. The application of these pastes was repeated two to three times in a year when they were washed off. The number of plants for each treatment was three except the control which had 10 plants. The date of complete girdling of the collar by the organism in case of control, the circumference of the collar at the point of inoculation, the maximum width of the scraped off portion and the width left uncovered by callus were recorded in November 1963 (Table 1). The data of death of the control plants as indicated by the drying of all the leaves of the plant were also recorded (Table 1).

TABLE 1 : Effect of various treatments on the control of collar rot of loquat.

Treatment	No. of plants	Circumference of collar (in cms.)	Date of complete girdling	Width of scraped off portion (in cms.)	Width left uncovered by callus in Nov. 1963	Date of death of the plant
1	2	3	4	5	6	7
Control	1	16.5	28.4.62	—	—	3rd week Sept. 62
	2	18.0	28.4.62	—	—	2nd week June 62
	3	19.0	28.4.62	—	—	2nd week June 62

Contd.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Contd.

1	2	3	4	5	6	7
Control	4	14.4	28.4.62	—	—	Plants did not die ; new growth of bark is coming up
	5	17.0	12.4.62	—	—	
	6	15.5	28.4.62	—	—	3rd week of Sept. 62
	7	20.5	21.4.62	—	—	1st week Aug. 62.
	8	22.0	28.4.62	—	—	2nd week Aug. 62.
	9	18.5	21.4.62	—	—	Plant did not die ; new growth of bark is coming up
	10	26.5	Complete girdling did not take place.	—	—	New growth of bark is coming up
Scraping the infected portion and application of Shell Copper paste	1	26.2	—	11.5	2.7	—
	2	23.1	—	11.5	1.2	—
	3	26.4	—	12.5	2.3	—
Scraping the infected portion and application of Bordeaux paste	1	26.5	—	14.5	5.6	—
	2	31.0	—	15.7	4.0	—
	3	26.0	—	12.5	4.1	—
Scraping the infected portion and application of Chevestelon solution.	1	33.8	—	12.3	3.0	—
	2	31.0	—	12.0	4.5	—
	3	31.0	—	10.9	4.0	—
Scraping the infected portion only without application of any paste.	1	19.5	—	11.5	3.5	—
	2	30.0	—	12.5	4.5	—
	3	27.3	—	12.5	3.6	—

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It is clear from the above table that all the control plants, except one, showed complete girdling of the collar by the organism in about a month from the date of inoculation. The girth of the collar varied from 14.4 to 26.5 cm. Six of the plants whose collars were completely girdled died between the following June to September, that is, in about 3 to 6 months from the date of inoculation and 2 to 5 months after the apparent complete girdling of the bark by the fungus. The remaining three control plants whose collars were apparently girdled, did not die. On close observation, it was found that a small patch of the bark was not infected deep into the tissue and only upper layer was infected. The callus from the remaining bark was putting forth new bark which was covering up the infected area from where the infected bark had sloughed off.

All the treated plants survived and new bark was observed to cover-up the scraped off area. The scraped off area of one of the plants to which shell copper paste was applied in March 1962 was completely covered up by new bark by November 1963.

DISCUSSION : It has been observed that the inoculations carried out with *Sclerotium* sp. often resulted in complete girdling of the collar and ultimate death of the plant. It was further observed that in the inoculated plants, the organism could be reisolated from the infected bark in the initial stages till complete girdling took place. However, in the later stages of infection, that is, by the time the plant died or was about to die, only *Diplodia* sp. was invariably isolated.

This leads to the conclusion that the collar-rot is initiated by *Sclerotium* sp., but in later stages *Diplodia* sp. also infects as a secondary organism and predominates in such a manner that *Sclerotium* sp. can hardly be isolated. This was corroborated by the cultural studies. When the two organisms were grown side by side on potato dextrose agar in a petridish, the quick growing, fluffy aerial mycelium of *Diplodia* sp. suppressed the slow growing *Sclerotium* sp. within 7 days. This apparently accounts for the isolation of *Diplodia* sp. from the collar of the dead plants in later stages.

Inoculations carried out at monthly intervals revealed that those carried out between March and June often resulted in complete girdling and ultimate death of the plant. Those carried out between July to September resulted in apparently complete girdling of the bark, but the plant was not killed, whereas the inoculations carried out between November and February neither gridled the branch nor penetrated deep into the bark and only the outer layer of the bark was infected.

The above observations may be explained in the following manner. There was a competition between the growth of the bark and that of the fungus and during March to June, the rate of growth of the bark was slow and conditions for rapid growth of the fungus were favourable and hence complete girdling took place and the plant died. But during July to September conditions for the rapid growth of the bark as well as the fungus were favourable which resulted in apparent complete girdling of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the branch but the inoculated plants did not die. However, close observations revealed that some portion of the bark usually remained uninfected which has put forth new growth and the plant was not killed. The new bark had come up from underneath and the dead one was sloughed off. Inoculations during November to February showed that the fungus did not penetrate deep into the bark and only the outer layer of the bark was partially infected. This can be explained on the basis that the growth of the fungus as well as the bark was slow during this period.

The control experiments indicate that even the treatments where the infected bark has been merely scraped off and no paste was applied, the bark is gradually covering up the scraped off area without fresh infection, but, it is better to apply the fungicidal paste as a preventive measure as the injured surface is more prone to infection.

SUMMARY

The collar-rot of loquat is initiated by *Sclerotium* sp. but *Diplodia* sp. also infects invariably as a secondary organism. The maximum, minimum and optimum temperatures for the growth of *Sclerotium* sp. is between 35-39°C, 12-14°C and 27—30°C respectively.

In order to control collar-rot, infected bark alongwith about 1 cm. of the healthy bark all round should be scraped off and Bordeaux paste, copper oxychloride paste or Chevestelon solution should be applied to the scraped off portion.

ACKNOWLEDGEMENT : The author wishes to express his sincere thanks to Dr. A. Prasad, Director, Horticultural Research Institute, for his keen interest and encouragement during the course of present investigations. Thanks are also due to Dr. T. N. Shukla, Virus Pathologist, for critically going through the manuscript.

Government Horticultural Research Institute,
Saharanpur, U. P. (India).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ASSAY OF BORDEAUX MIXTURE AS SOIL FUNGICIDE

O. P. SHARMA AND A. C. JAIN

(Accepted for publication July 3, 1965)

Bordeaux mixture was the first widely used fungicide and it is still recommended by many. Vaartaja (1964) has quoted quite a few references wherein promising results with copper compounds by treating soil have been reported. In India Bordeaux mixture application to soil as a drench for the control of foot-rot disease of *Pan* (Betlevine) due to *Phytophthora parasitica* has been recommended by various workers (Dastur 1931, Uppal 1936, Sundararaman 1932, Chowdhury 1944, Asthana 1947, Subramanian 1964). However, Asthana (1942, 1944, 1945) has reported mortality as high as 90.6 per cent in plots treated with Bordeaux mixture as against 100 per cent in control plots, giving only about 10 per cent control of the disease. Mahmud (1950) also found in pan gardens at Ramtek that inspite of regular soil treatment, foot-rot disease went on increasing from 1.2 per cent in the 1st year to 19.5 per cent in the 3rd year. These reports indicate that this fungicide was not much effective in checking the spread of the disease. In view of the above it was found necessary to test the efficacy of this fungicide as soil treatment for the control of soil borne pathogens. With this object in view the studies reported in this article were undertaken.

MATERIAL AND METHODS : The soil used in this experiment was the field soil which was first air-dried and sieved, then autoclaved at 15 lb./sq. inch for 45 minutes. It was then re-sieved before use. Bordeaux mixture used for drenching purposes was freshly prepared in the required strength. Dry Bordeaux mixture was obtained by filtering the freshly prepared 4 per cent Bordeaux mixture and oven drying the same at 80°C for 24 hours. Other proprietary fungicides used were obtained from the respective firms. The *Pythium* species used as test organism was obtained from the roots of wheat seedlings and *Alternaria* sp. the spores of which were used for slide test was obtained from leaf-spot of pan.

For testing the efficacy of applying the fungicide by drench method, glass tubes open at both ends (24 cm. long and 2.4 cm. in diam.) were used. The lower ends of these tubes were closed by muslin cloth in order to retain the soil column. A disc (10 mm. in diam.) was cut out from three day old cultures of *Pythium* sp. grown on rice agar and was placed in the tube on muslin cloth with its mycelial surface up. Then soil was introduced in the tubes to required height. These tubes were then suspended and a sterilized beaker was placed under each to collect the leachate. After this, 50 ml. of the freshly prepared solution/suspension of the test fungicide was allowed to leach through the soil column. After 4 hours the muslin cloth was removed and the fungal discs were taken out, washed in sterile water 3-4 times to remove the sticking soil particles and cultured on rice agar to test the viability of the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

fungus in those discs. Leachates from the tubes collected in beakers were tested for their fungicidal activity by slide germination test with *Alternaria* spores.

For testing the efficacy of different fungicides applied as soil dry mix, the sterilized and sieved soil and the required quantity of the fungicides were taken in Erlenmyer flasks and mixed thoroughly by shaking. This soil was then placed on mycelial disc in tube to the required height and instead of fungicidal solution/suspension 50 ml. of sterilised water was added slowly. The rest of the procedure was the same as that in the fungicidal drench test.

EXPERIMENTAL RESULTS : 1. *Assay of Bordeaux mixture and other fungicides by tube test for their efficacy as soil fungicides* : This experiment was conducted in tubes. In one set the fungicide was applied by drench method and in another as a dry-mix. The efficacy of each treatment was judged by its action on the viability of the test fungus (*Pythium*) in the discs. There were three replications of each treatment and the experiment was repeated twice with the same results.

TABLE 1 : Efficacy of Bordeaux mixture and other fungicides applied to soil in tubes.

Treatment		Death of the Pythium fungus in disc. Discs placed below soil		
		1″	2″	3″
Drench method				
1. Bordeaux mixture	0.4%	—	—	—
2. Bordeaux mixture	2.0%	—	—	—
3. Cupravit	1 0%	—	—	—
4. Dithane Z-78	1.0%	—	—	—
5. PCNB	1.0%	—	—	—
6. Agallol	0.5%	+	+	+
7. Ceresan wet	0.2%	+	+	—
8. Copper sulphate	0.4%	—	—	—
9. Control (No fungicide)		—	—	—
Soil dry mix method				
1. Bordeaux mixture 4000 ppm of copper sulphate		+	+	+
2. B. mixture 2000 ppm of $CuSO_4$		—	—	—
3. Ziram 2500 ppm		+	+	+
4. Ferbam ,, ,,		+	+	+
5. Thiuram ,, ,,		+	+	+
6. Dithane Z-78 2500 ppm		+	+	+
7. PCNB ,, ,,		—	—	—
8. Control (No fungicide)		—	—	—

(+) Effective for killing test fungus.
(—) Ineffective for killing test fungus.
PCNB Pentachloro-nitrobenzene.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The data in the table 1 show that Bordeaux mixture and other fungicides that form suspension in water do not penetrate soil and remain on its surface. The water soluble fungicides (Agallol and Ceresan wet) penetrated the soil column. Agallol was found in the leachate after penetrating 3" soil column whereas Ceresan-wet penetrated more than 2 inches but not 3 inches of the soil column. Copper sulphate, though soluble in water did not penetrate even one inch of soil indicating that the copper sulphate molecules are strongly adsorbed on the soil particles. The leachates tested by spore germination test also confirmed the above findings.

Bordeaux mixture as a dry mixture equivalent of 4000 ppm. of copper sulphate killed the test fungus but at 2000 ppm. of copper sulphate the test fungus was not killed. Ziram, Ferbam, Thiuram and Dithane Z-78 at the rate of 2500 ppm. as dry mixture in soil also killed the test fungus while PCNB 2500 ppm. failed to do so.

DISCUSSION AND CONCLUSION : In these studies where experiments have been made to find the effect of fungicides after penetrating a certain column of soil, tubes open at both ends, the lower end closed by muslin cloth, have been used. This is an improvement on the vial method used by Zentmyer (1955) because in this case the soil-air escapes down through the muslin cloth without disturibing the soil column and giving any chance to the fungicide solution reaching down through pores made by air bubbles. This avoids the chances of error regarding observations about penetration capacity of the fungicide under trial.

This technique has the further advantage that the fungal mycelial disc can easily be taken out and the leachate obtained can be separately assayed for fungicidal property.

Soil columns are perfect filters. Bordeaux mixture is a suspension hence the fungicide particles remain on the soil surface. Further the Cu-ions which may be released slowly also cannot penetrate the soil to any depth as it has been found that Cu-ions (added as $CuSO_4$—solution to the soil) are strongly adsorbed by the soil colloids. Thus the use of Bordeaux mixture as soil drench to control the soil borne pathogens is limited to those organisms which are on the soil surface. The pathogens found below the surface are not affected as the copper does not penetrate to any depth. Probably this may be the reason why Bordeaux mixture as soil treatment for foot-rot of pan did not give effective control.

Bordeaux mixture used as dry mixture is effective only upto the depth it is mechanically mixed but the thorough dry mix application on field scale is impossible. Further the effective dose required is 4000 ppm. of copper sulphate equivalent which is both uneconomical as well as toxic to the crops.

Other fungicides which form the suspension (Ziram, Thiuram etc.) also don't penetrate the soil column and therefore they are not effective against pathogens below soil surface.

The mercurial fungicides which form solution were found to penetrate the soil column. When applied as drench, Agallol 0.5 per cent

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

solution and Ceresan-wet 0.2 per cent were effective in killing the test organism placed below 3″ and 2″ soil respectively. The possibility of their application as soil fungicides needs further investigation.

SUMMARY

The technique used for testing Bordeaux mixture and other fungicides as drench and dry mix. in tubes has been described. It has been found that fungicides forming suspension remain on the soil surface when applied as drench and Cu-ions even in solution are adsorbed by soil colloids. However the organomercurials soluble in water were found effective against pathogens upto 2 to 3 inches below soil surface.

Bordeaux mixture is effective as soil fungicide if used as dry mix at 4000 ppm. of copper sulphate equivalent but this technique on field scale is difficult to carry out and the effective dose is both uneconomical as well as toxic to crops.

Plant Pathology Section,
Jawaharlal Nehru Krishi Vishwa Vidyalaya,
Jabalpur.

REFERENCES

ASTHANA, R. P. (1942 to 1945) Report, Pan cultivation scheme C. P. 1941-42, 24 pp., 1943-44, 16 pp, 1944-45, 18 pp.

————————(1947) Diseases of *Piper betle* and their control. *Indian Farming* **8** : 394-397.

CHOWDHURY, S. (1944) Diseases of Pan in Sylhet, Assam, Part II. *Proc. Indian Acad. Sci.* **14** (B) : 152-164.

DASTUR, J. F. (1931) Control of foot-rot disease of Pan (*Piper.betle*) in the Central Provinces. *Agric. and Live Stock in India.* **1** : 26-31.

MAHMUD, K. A. (1950) Manurial requirements of Pan (*Piper betle*) on morand soil in Madhya Pradesh. *Nagpur Agric. Coll. Mag.* **25** : 13-17.

SUBRAMANIAN, K. S. AND A. VENKATA RAO (1964) Some experiments on betlevine wilt at Pothanur, Madras State. (Abs.). *Madras Agric. Jour.* **51** : 96.

SUNDARARAMAN, S. (1932) Adm. Rept. Govt. Mycologist, Madras 1931-32, 17 pp.

UPPAL, B. N. (1936) Leaf-let No 7 of 1936. *Dept. Agric.*, Bombay.

VAARTAJA, O. (1964) Chemical treatment of seed beds to control nursery diseases, *Bot. Rev.* **30** : 1-91.

ZENTMYER, G. A. (1955) A laboratory method for testing Soil fungicides with *Phytophthora cinnamomi* as test organism. *Phytopathology* **45** : 398-404.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE EFFECT OF STEM RUST ON THE UPTAKE AND INCORPORATION OF P^{32} BY WHEAT SEEDLINGS

K. L. MUKHERJEE* AND MICHAEL SHAW

(Accepted for publication July 26, 1965)

INTRODUCTION : In an earlier communication (Mukherjee and Shaw, 1962) we showed that rust infected leaves of Little Club wheat have higher concentration of phosphate and a lower ratio of inorganic to organic phosphate than uninfected leaves. Work in several laboratories has shown that when P^{32} is fed to rust infected plants, it accumulates at the sites of the rust infections (Shaw, 1963). In the present report, using P^{32} as tracer, we have illustrated the pattern of phosphate uptake by rust infected wheat seedlings and incorporation of radioactivity into various organic phosphate fractions.

MATERIAL AND METHODS : Little Club wheat was grown in 6" pots filled with sand. Seedlings were inoculated with *Puccinia graminis tritici* Erikss. and Henn. (Race 15B) as described earlier (Mukherjee and Shaw, 1962). Infection flecks became visible by the 5th day and sporulation began on the 9th day after inoculation. All analyses were carried out using first seedling leaves, each of which developed from 20-40 sporulating pustules.

For feeding P^{32}, wheat seedlings were first removed from the sand and the roots were thoroughly washed in tap water to remove adhering sand particles. Bunches of 5-20 seedlings, wrapped in loose cotton plug, were transferred to a 500 ml. Erlenmeyer flask with the roots immersed in 250 ml. Hoagland's solution with micronutrients (Meyer and Anderson, 1952). A long-stem thistle funnel through the cotton plug was used to introduce P^{32} (added as ammonium phosphate) into the nutrient solution. Water was also added periodically through this funnel to maintain the level of the nutrient solution in the flask. Plants were allowed to take up P^{32} under normal greenhouse conditions.

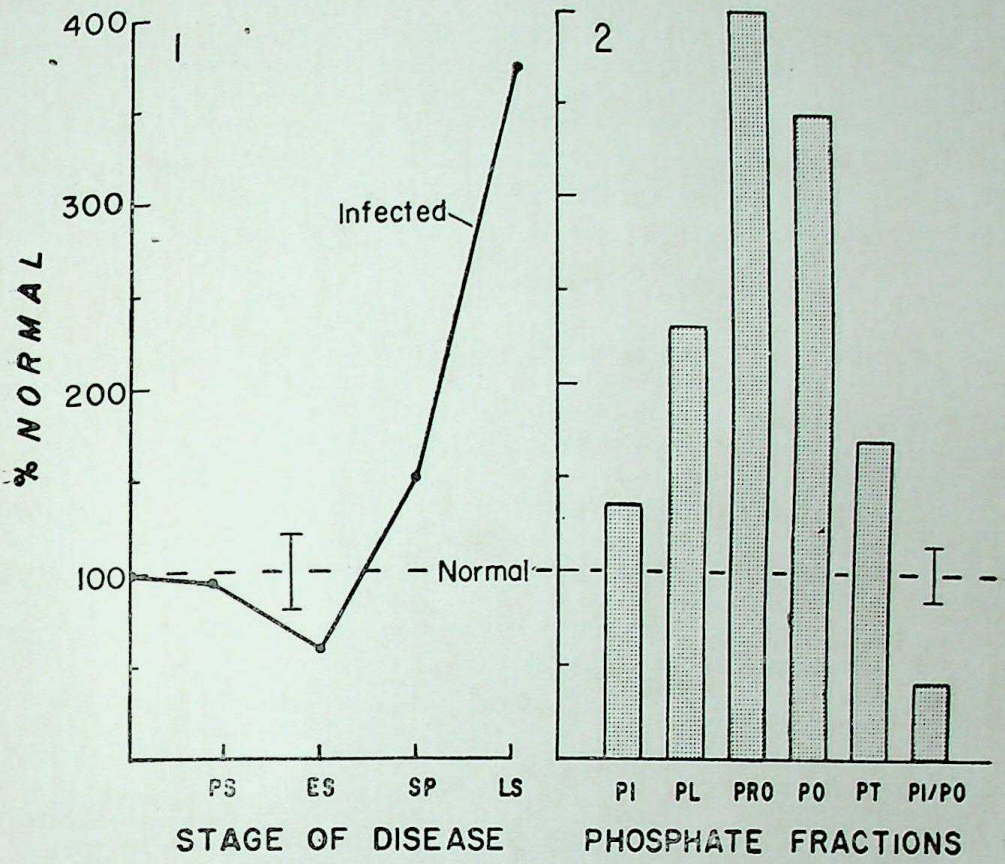
EXPERIMENT I : Wheat seedlings were transferred to the radioactive solution (0.01 μc P^{32}/ml.) on the 4th. day after inoculation and P^{32} was available continuously thereafter until the 13th day after inoculation. The nutrient solution containing P^{32} was replaced twice (7th and 10th day after inoculation) during the course of the experiment. Samples of first leaves were taken on the 5th day (presporulation), 9th day (early sporulation), 11th day (sporulation) or on the 13th day (late sporulation) after inoculation. Total radioactivity of P^{32} accumulated in the first leaves was determined after drying the leaves for 24 hours at 80°C and grinding to a fine powder. The powder was spread as uniformly as possible on a tared stainless steel planchet, weighed and the radioactivity measured in a gas flow counter.

EXPERIMENT II : Wheat seedlings were transferred at the sporulation stage (11th day) and allowed to stay in the radioactive solution (o.50 μc P^{32}/ml.) for 24 hours only. Subsequent to this, phosphate fractionations were done according to Mukherjee and Shaw (1962). Two ml. aliquot of each fraction was evaporated to dryness in planchets under an infra-red lamp and the radioactivity measured in a gas flow counter.

*Present Address : Dept. of Botany, Banaras Hindu University, Varanasi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 1—2. 1. Accumulation of P^{32} in the first seedling leaf of Little Club Wheat at various stages of rust infection. Presporulation (PS), early sporulation (ES), sporulation (SP), late sporulation (LS); samples taken on the 5th 9th, 11th and 13tn day respectively after inoculation. 2. Incorporation of P^{32} into various phosphate fractions at the sporulation stage of rust infection. Inorganic (PI), lipid (PL), acid insoluble or residual organic P (RO), total organic (PO) and total (PT) phosphates ; ratio of inorganic/organic phosphates (PI/PO). Bars on Figs. 1 and 2 represent significant differences at 5 per cent level.

RESULTS : Fig. 1 (Experiment I) shows that with the beginning of sporulation (5 days after inoculation) uninfected first leaves accumulated more P^{32} than the infected ones. After sporulation (9 days after inoculation) this situation was reversed and that the infected leaves accumulated more than 350 per cent of P^{32} activity than the normal.

Fig. 2 (Experiment II) shows that an increased level of P^{32} occurred in both inorganic as well as in the organic phosphate fractions and much larger amounts were incorporated into the organic phosphate fractions of the rust infected leaves than the uninfected ones. Particularly striking increases occured in the P^{32} activities of phospholipids (PL), total organic phosphates (PO) and acid insoluble organic phosphates (PRO) of the infected leaves. The PI/PO ratio of the infected leaves fell to less than one-half of the normals.

DISCUSSION : During the initial phase of rust infection, the normal phosphate assimilation rate of the first seedling leaf of wheat does not

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

seem to be affected. During the sporulation stage, rapid biochemical changes occur in the infection sites demanding a greater uptake of phosphate through the host. The fate of the newly assimilated phosphate is obviously in the building up of reserve organic phosphates. The striking fall of PI/PO ratio suggests a high synthetic activity in the host-parasite complex. The findings of Rohringer and Heitefuss (1961), Heitefuss (1961), Heitefuss and Fuchs (1961, 1963) support this conclusion.

SUMMARY

Rust infection increased uptake of P^{32} by wheat seedlings. Radioactivity increased more in the total organic phosphates than in the total inorganic phosphate fraction of infected leaves. This fall of PI/PO ratio was indicative of increased synthetic activity in the host-parasite complex.

ACKNOWLEDGEMENT : We acknowledge with gratitude the help of Dr. E. Riley, Department of Radiation Biology, State University of Iowa, Iowa City, USA, for a critical review of this work.

This project was supported by a grant to Dr. M. Shaw from the National Research Council of Canada.

Department of Biology,
University of Saskatchewan,
Saskatoon, Sask, Canada.

REFERENCES

HEITEFUSS, R. (1961) Untersuchungen uber den Phosphatstoffwechsel von Weizenkeimpflanzen unterschiedlicher Resistenz gegen Schwarzrost mit Hilfe von P^{32}. *Ber. Deut. Botan. Ges.* **74** : 359-369.

HEITEFUSS, R. AND W. H. FUCHS (1961) Untersuchungen zum Phosphatstoffwechsel von Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici*. *Naturwissenschaften* **48** : 505-506.

HEITEFUSS, R. AND W. H. FUCHS (1963) Phosphatstoffwechsel und Sauerstoffaufnahme in Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici*. *Phytopath. Z.* **46** : 174-198.

MEYER, B. S. AND D. B. ANDERSON (1952) *Plant Physiology*, D. Van Nostrand Co., Inc., New York.

MUKHERJEE, K. L. AND M. SHAW (1962) The physiology of host-parasite relations, XI. The effect of stem rust on the phosphate fractions in wheat leaves. *Can. J. Botan.* **40** : 975-985.

ROHRINGER, R. AND R. HEITEFUSS, (1961) Incorporation of P^{32} into ribonucleic acid of rusted wheat leaves. *Can. J. Botan.* **39** : 263-267.

SHAW, M. (1963) The physiology and host-parasite relations of the rusts. *Ann. Rev. Phytopath.* **1** : 259-294.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MUTATION IN COLLETOTRICHUM FALCATUM, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE-VI. INDUCED BIOCHEMICAL MUTANTS AS TEST ORGANISMS FOR BIOASSAY OF P-AMINOBENZOIC ACID AND BIOTIN

B. S. BAJAJ*, M. S. CHATRATH AND R. S. VASUDEVA

(Accepted for publication July 26, 1965)

The biochemical mutants of *Colletotrichum falcatum* Went, the red rot organism of sugarcane induced as a result of irradiation with P^{32} in growth phase showed specific growth response to the addition of vitamins like p-aminobenzoic acid, biotin, thiamine, etc. in the minimal medium, thus indicating the possibility of utilising some of these mutants as test organisms for microbiological assay of respective growth factors.

Mutants-X_{11} and V_1 (Bajaj, Chatrath and Vasudeva, 1964) showing deficiency for PABA and biotin, respectively, were selected for this purpose because of their specificity and stability in respect of vitamin requirement. Inoculum, adjusted at approximately 15×10^4 spores/ml. with previously washed spores of 10—15 days old cultures, was used at the rate of 0.1 ml. for conducting growth studies in 15 ml. liquid media in each of the 100 ml. Erlenmeyer flask. Growth was recorded as an average dry mycelial weight of six replicates after incubation at 25°C for 10 days. Dextrose asparagine-phosphate (DAP) medium consisting of 30 g. dextrose, 1.0 g. asparagine, 0.5 g. $MgSO_4$ $7H_2O$, M/30 Sorensen's phosphate buffer (pH 6.0) per litre, was used as basal medium.

For all the experiments, acid washed pyrex glassware, A. R. grade (Analar) chemicals, glass distilled water and muslin-protected cotton plugs were used. Media were sterilised at 10 lb. p. s. i. pressure for 10 minutes.

Optimum concentration of PABA and biotin for the growth of the two biochemical mutants was found to be around 5×10^{-2} μg/ml. and 2×10^{-3} μg./ml., respectively but these mutants showed growth response at as low a concentration as 5×10^{-4} μg./ml. of PABA and 2×10^{-6} μg./ml. of biotin which showed that these mutants were quite sensitive to the presence of even traces of the vitamins in the medium. In both the cases, the response between log concentration of the vitamins and growth of the organisms appeared to be linear with in certain ranges (Fig. 1).

In order to develop a suitable rich medium, for bioassay of these vitamins, keeping in view the general requirements of assay media (Lilly

*Present Address :—Deputy Superintendent, Antibiotics Project, Virbhadra (Rishikesh).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

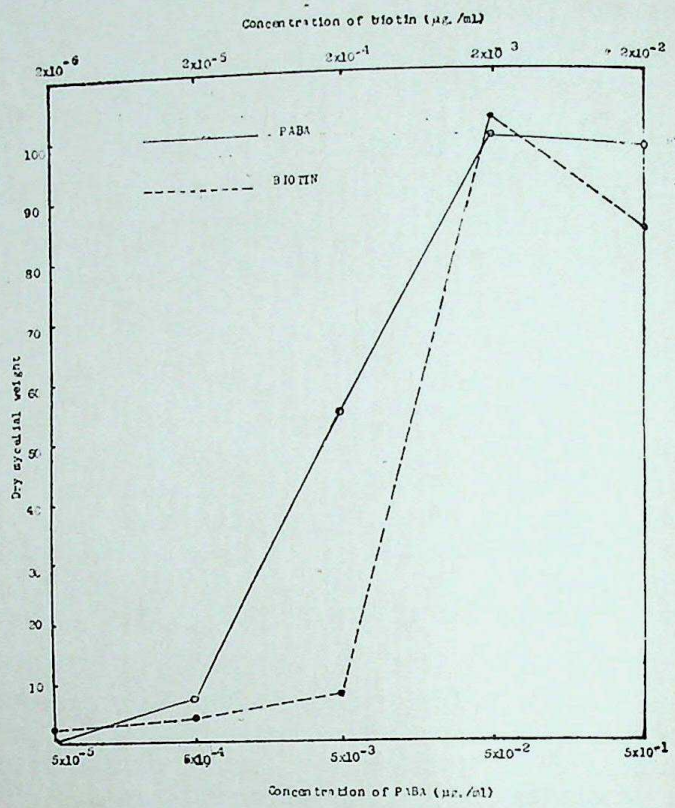


Fig. 1 Effect of different concentrations of PABA and biotin on the growth of the mutants X_{11} and V_1 of *C. falcatum*.

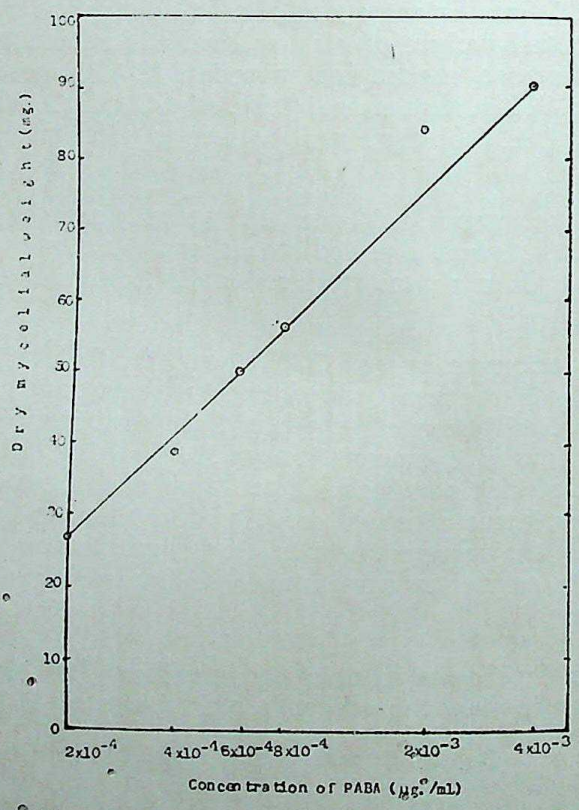


Fig. 2 Standard bioassay curve of the biochemical mutant (X_{11}) of *C. falcatum*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and Barnett, 1951), the basal medium, DAP was supplemented with different nutrients, using optimal concentrations of PABA and biotin. In treatments where mixture of vitamins was incorporated, the concentrations of different vitamins were same as reported earlier (Chatrath and Bajaj, 1964). The results (table 1) showed that DAP medium containing vitamin mixture and casein and DAP medium supplemented with mixture of trace elements were suitable for the mutants X_{11} and the mutants V_1, respectively.

TABLE 1 : Effect of various amendments of DAP medium on the growth of the mutant strains of *C. falcatum* deficient in p-aminobenzoic acid and biotin.

Medium	Average dry mycelial (weight) (mg.)	
	Mutant X_{11}	Mutant V_1
DAP	0.0	0.0
DAP+GF*	83.8	102.1
DAP+vitamin mixture	104.9	101.5
DAP+GF+Casein	98.1	115.1
DAP+GF+trace elements	—	125.6
DAP+Vitamin mixt.+Casein	105.9	—
DAP+vitamin mixture +casein+trace elements	—	115.5

*GF indicates PABA in the case of mutant X_{11} and biotin in case of mutant V_1.

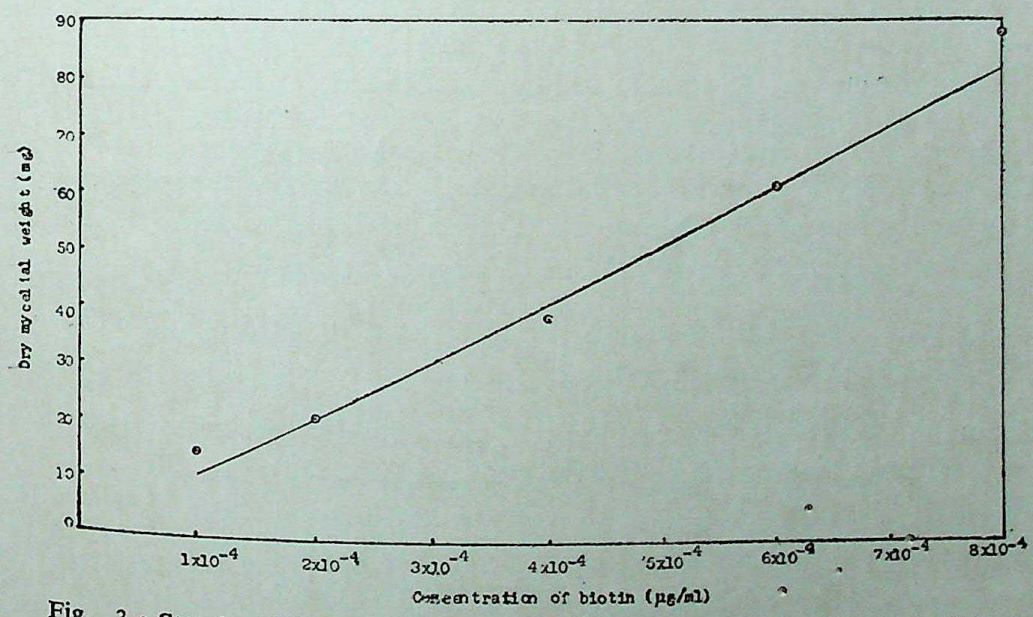


Fig. 3 : Standard bioassay curve of biotin using the biochemical mutant (V_1) as test organism.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Utilizing the above media, experiments for the standard curves for bioassay of PABA and biotin, employing the mutants X_{11} and V_1, as test organisms were set up with narrow range concentration of PABA and biotin and the results are given in figs. 2 and 3.

A linear relationship was obtained between the graded concentrations of PABA ranging from 2×10^{-4} to 4×10^{-3} μg./ml. and that of biotin ranging from 1×10^{-4} to 8×10^{-4} μg./ml. and the growth of the mutants X_{11} and V_1, respectively. The mutants were maintined on artificial medium for more than 4-5 years without any indication of revesion or change in any other character showing thereby that these are quite stable genetically. Both these mutants showed absolute deficiency as they did not show any trace of growth on media devoid of the respective growth factor. All these characters coupled with the linear response of growth with the graded concentrations of the two vitamins and the sensitivity of the mutants adequately satisfy all the conditions putforth by snell (1950) and Lilly and Barnett (1951) for bioassay of growth factors and thus show the possibility of utilising these mutants as test organisms for bioassay of PABA and biotin.

ACKNOWLEDGEMENT : The authors are thankful to Dr. B. L. Chona, Head of the Division for critically going through the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

BAJAJ, B. S., M. S. CHATRATH AND R. S. VASUDEVA (1964) Mutation in *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane IV. Morphological and biochemical mutants induced by ionizing radiation. *Indian Phytopath.*, **17** : 287-294

CHATRATH, M. S. AND B. S. BAJAJ (1964) Studies on the physiology and pathogenicity of an avirulent strain of *Colletotrichum falcatum*. *Indian Jour. Sugarcane Res. and Develop.* **8** ; 288-292.

LILLY, V. G. AND H. L. BARNETT (1951) Physiology of fungi. McGraw-Hill Book Co. Inc. New York.

SNELL, E. E. (1951) Microbiological methods in vitamin research. Vitamin Methods. Ed ; Gyorgy Paul, **1** : 327-505.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A PATHOGENIC CUNNINGHAMELLA ON PUMPKIN AND ITS CONTROL

RAJENDRA K. GROVER

(Accepted for publication July 29, 1965)

A severe disease of rotting of young developing fruits of Spanish pumpkin (*Cucurbita maxima* L.) observed at Chandigarh and 3 neighbouring villages in Punjab in July-August, 1961, appeared to be caused by *Cunninghamella echinulata* Thaxter. This organism has not been reported to be pathogenic, but has been frequently isolated from soil from different parts of the world (Gilman, 1957). Neklyudova (1960) reported a disease complex of young pumpkin fruits caused by species of *Pythium* and *Rhizopus* which produced symptoms very similar to the disease caused by *Cunninghamella*. The present account records the symptoms of the disease, its pathology, etiology, and the physiology of the pathogen along with some control measures.

SYMPTOMS : The disease begins to appear only after flowering of the plants, i. e. the fourth and sixth week after planting. Symptoms are first recognizable when the flowers begin to die off gradually. The healthy flowers normally open early in the mornings and close by afternoon. But the diseased flowers do not open properly, or may not open at all. On dissecting such un-opened flowers, cottony mycelial growth is invariably seen around the base of staminal or pistilate tube. Conspicuous blight symptoms appear after 3-4 days and the flowers fail to reopen and eventually drop down, converting the affected tissues into a soft rotting mass. A dense coating of whitish conidial heads become visible on the rotted regions (Fig. 1). In the pistilate flowers with the advance of time and favourable conditions the rot extends from top-end of the young ovary to the pedicel-end rapidly. The petals fall and soon the young fruits turn dark brown and are covered extensively with cottony mycelial growth bearing numerous conidial heads (Fig. 2, 3). The affected fruits may get detached from the plant and continue to rot on the soil, or, the rotted fruits may remain attached to the stalk at the onset of warm and dry season and become shrivelled and dry, representing a mummified condition. Under favourable conditions the rot may even extend to some length to the stalks also, but in no case the trailing stem has been found to be infected in the field.

Very frequently, the leaves are also found to be affected (Fig. 4). Usually the infections commence from the margins and then extend inwards; rarely small irregular water soaked lesions appear on the leaves at regions other than the margins. Initially the affected regions are smaller, but gradually extend all around encircling a larger area. The infected portions turn brown in the center leaving a chlorotic area around. The lesions are irregular in shape and size, and may involve

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

whole leaf under favourable conditions, which may further extend down to the petioles. Under dry conditions the invaded regions become restricted.

ISOLATION AND PATHOGENICITY TESTS : Diseased tissues showing typical symptoms in all stages of development were collected from 5 pumpkin fields. *C. echinulata* was isolated from all diseased parts of the plant on acidified 2 per cent potato dextrose agar medium by the usual methods. Direct isolation from conidial heads growing on rotted tissues was also successful. The 5 isolates from different localities were purified by the single spore method and compared. No morphological differences were observed among the different isolates.

The pathogenicity tests were carried out in 3 experimental plots during July-August, 1962, in the field. A spore suspension, made in sterile water from 4-5 days old cultures growing on PDA had about 1×10^6 spores/ml., was sprayed early in the morning on the opened flowers, young fruits, leaves and stem. In each of the plots about 50 of each organs of the plants were sprayed and tagged. The 3 plots were separated from each other by a large distance. Half of the plant parts thus inoculated in each plot were covered with transparent cellophane bags in order to ensure high humidity for infection while the other half were left as such. Daily observations on the progress of the disease were made. The weather conditions during the period after inoculation remained favourable as the maximum temperature remained around $28 \pm 2°C$, and it remained mostly cloudy for the first 3 days, and on the next day there was slight precipitation, which further increased the humidity. The results of pathogenicity tests are given in Table 1.

TABLE 1 : Infection of flowers, young fruits, leaves and stem of *Cucurbita maxima* in 3 fields by *Cunninghamella echinulata* spores.

Plant organs inoculated	Per cent organs infected[1]		Days required for rotting	
	Uncovered	Covered	Uncovered	Covered
Flowers—open	100	100	3	2
—closed	80	96	5	3
Young fruits	84	100	4	3
Leaves	78	94	6	4
Stem	28	45	7	5

[1]Average of 3 plots, each plot having 50 different plant organs of the plant artificially inoculated.

The open flowers and young fruits are the most susceptible parts of the plant while the stem parts were infected slowly and with difficulty.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The progress of the disease accelerated under high humid conditions since different plant organs covered with cellophane bags rotted earlier than the uncovered ones. In all cases on re-isolation from different diseased plant parts typical *C. echinulata* was obtained.

Inoculations of different plant parts were also tried with vigorously growing 5-6 day old mycelial bits free from spores. In flowers the mycelium invaded the staminate tube or pistals after 6-8 days, while with spores the rotting occurred in 2-3 days.

When the mycelium of the fungus was traced in the diseased tissues, it was found to occur intercellularly and no haustoria were observed. The cortical parenchymatous cells were first invaded and advance rotting of cells extending in all directions was commonly observed, which was perhaps due to the release of pectolytic enzymes secreted by the organism.

MORPHOLOGY OF THE CAUSAL ORGANISM : The fungus in culture produces white, fluffy mycelium with abundant conidial heads. Zygospores were not observed under any conditions tried. Hesseltine (personal communication) feels that this strain is weak sexually, for it did not form any zygospores with other mating types of this species.

Mycelium typically coenocytic, becoming septate with age, and branched. Conidiophores erect, more or less irregularly branched-cymose to indefinite, or unbranched, bearing conidia upon terminal variously shaped, aerolate, spherical to obovoid vesicle. Conidiophores 5-8 mm in length, arise from the vegetative hyphae. From each capitate vesicle one to many small sterigmata arise, each of which in turn bear small finely echinulated, unicellular, globose to oval deciduous conidia measuring 8-18 $\times$ 10-24 μ.

The genus *Cunninghamella* was proposed by Matruchot (1903) and was placed in the order Mucorales near *Choanophora*. Lender (1908) in his systematic treatment of the genus placed it in the family Chaetocladiaceae. Later in 1939 Naumov proposed the family Cunninghamellaceae which was followed by Hesseltine (1955), and separated it from Choanophoraceae as suggested by Fitzpatrick (1930) and Zycha (1935).

The type culture has been deposited at ARS Culture Collection, Peoria, Ill., U. S. A. (No. A-12080) and will also be deposited at the Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi.

PHYSIOLOGICAL STUDIES : *Growth and sporulation on different media* : To determine the influence of various media on vegetative growth and sporulation, the following media were tried : PDA ; Oat meal agar ; Malt agar ; Czapek's synthetic agar ; Richard's synthetic agar ; and Brown's synthetic agar. Each of the 3 replicate petriplates containing 25 ml. of the medium were planted with a 5 mm. disc of agar bearing the fungus and all the petriplates were incubated at 28$\pm$1°C. The growth rate was measured daily. Sporulation was measured of a

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spore suspension made from a petriplate after 6 days growth diluted to 100 ml. water, and their number was calculated with the help of Heamocytometer. The results are given in Table 2.

TABLE 2 : Growth and sporulation of *Cunninghamella echinulata* on six media after 6 days at 28±1°C.

Basal medium	Diameter of colony (mm.)	Sporulation (1×10^6)
Potato dextrose agar	98	188
Oat meal agar	88	178
Malt agar	84	168
Czapek's synthetic agar	72	112
Richard's synthetic agar	76	116
Brown's synthetic agar	68	108

The data obtained concerning the growth characteristics of the fungus on the different media listed indicated that the organism could grow and produce spores on all the media. Semi-synthetic media, like PDA, favoured growth and sporulation more than the synthetic media. Since the growth was slower on synthetic media, accordingly, the sporulation was also relatively less.

Effect of H-ion concentration : For determining the effect of H-ion concentration on growth of the fungus, Richard's synthetic medium buffered and adjusted with 1.0 N HCl or NaOH to obtain the required pH was used. The pH range tested was from 2 to 10. Three flasks for each pH were used and inoculated with 1 ml. spore suspension containing 1×10^3 spores. After 5 days of incubation the mycelial mats were harvested, washed, dried and weighed.

It was seen that some growth was initiated at pH 3.0, while there was no growth at pH 2.0 and 9.0 (Fig. 5). A gradual increase in mycelial dry weight was seen upto pH 6.0, after which it began to decline. The sporulation was seen in all cases, but at lower and higher pH it was less.

The germination of conidia in different H-ion concentrations was studied in Richard's synthetic medium adjusted to different pH values. Five to 8 days old cultures were washed to obtain a spore suspension. The spores were washed twice by centrifugation and suspended in different pH solutions to give a final concentration of about 1×10^3 spores/ml. A drop of conidial suspension was put on a slide and kept in moist chamber. Three replicates of paired drops were examined for each pH after every 2 hours, and final readings were taken after 24 hours, after which no increase in germination was observed.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

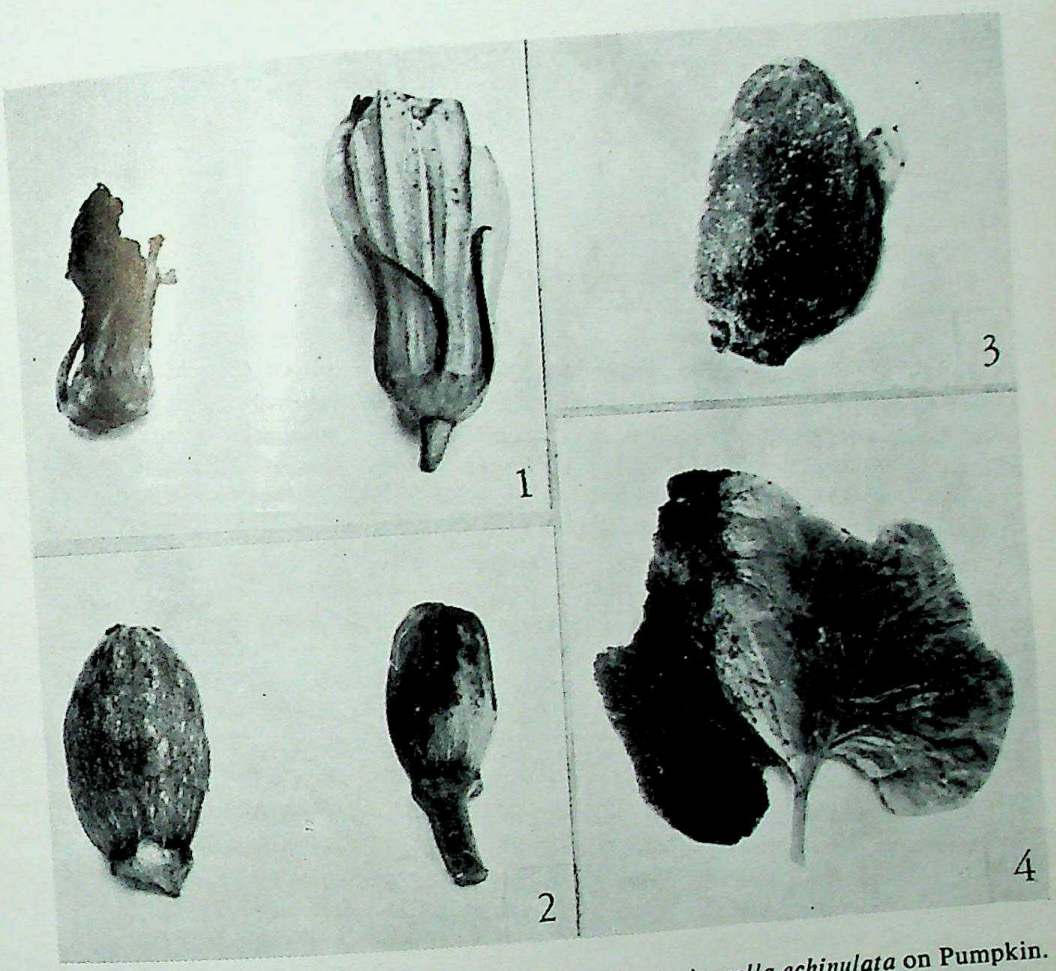


Fig. 1—4. Disease symptoms caused by *Cunninghamella echinulata* on Pumpkin.

1. Left—Rotted flower ; Right—Healthy flower.
2. Left—Healthy young fruit ; Right—Young fruit beginning to show browning from top region.
3. Rotted young fruit showing dense mycelial growth with conidial heads.
4. Affected leaf showing browning on the left side.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

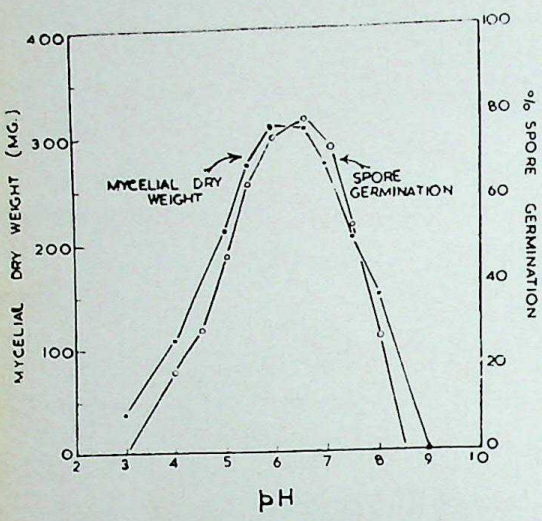


Fig. 5

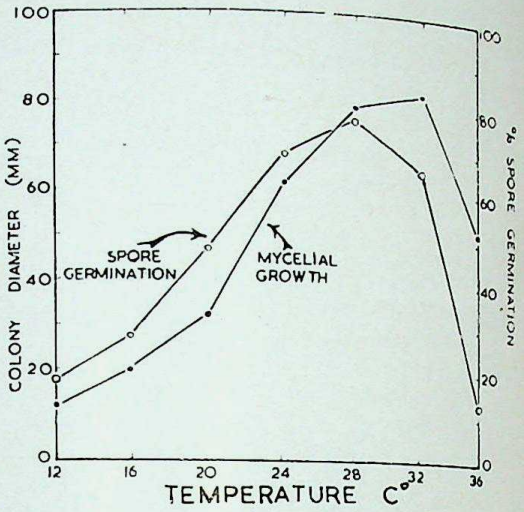


Fig. 6

Figs. 5—6. Effect of pH on growth and spore germination of *C. echinulata*. 6. Effect of temperature on growth and spore germination of *C. echinulata*.

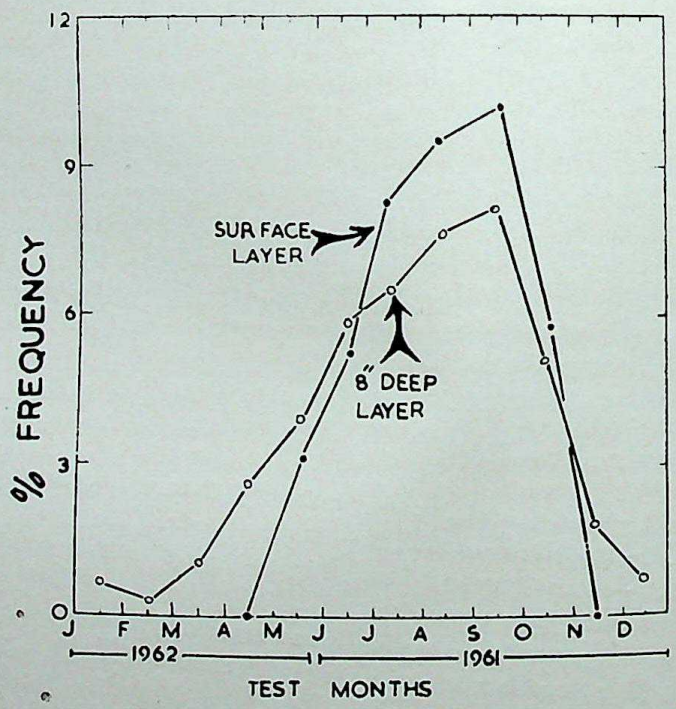


Fig. 7

Fig. 7. Frequency (per cent) isolation of *C. echinulata* from different soils at the surface layer and 8" deep layer.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Maximum germination of conidia was obtained at pH 6.5, while there was no germination at pH 3.0 and above 8.5 (Fig. 5). The germ tubes were relatively longer and often branched at pH 5-7, while at pH 4 and 8 and above, these were smaller, unbranched and appeared to be tougher. The conidia began to germinate after 3-4 hours at pH 5.5-6.5 and the germination was completed in 10 hours. At other pH values the conidia took 6-12 hours to initiate germination.

Effect of temperature : The culture of the fungus was grown in triplicate on PDA adjusted to pH 6.0 at constant temperatures ranging from 12-36°C with 4° increment. The rate of growth was determined by radial measurements and data obtained is presented in Fig. 6. The optimum growth and sporulation was at 28-32°C, while there was little growth at 12 and 36°C. The colonies were relatively thinner at lower temperatures. The sporulation was scanty at below 16°C and at 36°C.

Effect of temperature on spore germination was also noted by the method outlined earlier. Optimum spore germination was obtained at 24-32°C, while there was silght germination at 12°C and 36°C (Fig. 6).

Effect of humidity on spore germination : Clayton's (1942) method of maintaining different humidities in closed chambers was used for studying the effect of moisture on conidial germination. Dry spores obtained from 5-6 days old cultures by camel hair brush were dusted on to the slides, which were kept in chambers with different humidities. It was observed that the conidia germinated at a relative humidity of 32 per cent and the germination increased with the increase in relative humidity, reaching an optimum at 92-100 per cent R. H. No germination was seen at below 32 per cent R. H.

ETIOLOGY OF THE DISEASE : *Occurrence of the pathogen in soil* : *C. echinulata* has been frequently isolated from soil from different parts of the world (Gilman, 1957). Since the rotted flowers, fruits and leaves fell on the ground, it was assumed that this pathogen might survive in soil during the absence of the crop. Experiments were designed to analyze the soil samples from 3 localities where this disease was found to occur.

The soil samples were collected from each locality in sterile containers by using standard sterile borer from the surface layer and at soil depth of 8 inches over which the crop was growing. Four soil samples were collected from each of the 3 sites monthly. Warcup's (1951) soil plate method was used for isolating *C. echinulata*. Warcup's agar medium containing Agrimycin (1.0 μg./ml.) was used for all isolations. Agrimycin checked the growth of various bacteria and yeasts without affecting *C. echinulata*. Five replicate petriplates containing Warcup's medium were used for each of the soil sample. Colonies of *C. echinulata* were noted after 2-4 days incubation, and after 6 days the total number of these colonies were counted and the results are expressed in terms of percent frequency, calculated as below :

$$\text{Per cent frequency} = \frac{\text{Sites of occurrence}}{\text{Total sites sampled}} \times 100$$

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Such analyses were made once every month during the 2nd week beginning from June, 1961 to May, 1962, and the results are presented in Fig. 7.

It was observed that the frequency isolations of the pathogens on the soil surface layer was in relatively larger proportions during the crop season, and after a month of harvest the fungus disappeared. At the 8″ deep layer the fungus could be isolated from soil all the year round and there was a continuous increase in its frequency as well as population from February till little after harvest. It appeared that with the onset of sowing the frequency and population of the fungus continued to increase untill the harvest time. Since the soil became denuded after harvest of the crop and due to unfrequent precipitation, the existing spores of the fungus on or in the soil died, thus resulting in a rapid decline in population and frequency during the months of October-November. Random isolates from soil were tested for pathogenicity and it was found that all the isolates were pathogenic.

Dried mummies as source of infection : A large number of dried up mummified young fruits were collected from field before harvest and stored in the laboratory for the analysis of survival of the pathogen. Periodically these mummified furits were checked for the viability of the fungus by putting surface sterilized dried up pieces (3-4 mm.) on acidified potato dextrose agar medium. It was possible to obtain the pathogen from these mummies upto 32 months, and even at this stage there seemed to be no loss of viability of the fungus. Abundant conidial heads appear on these dried up bits after 4-6 days. Besides, when these mummified pieces were placed in humid chambers, profuse mycelial growth and sporulation appeared on these parts.

ROTTING OF YOUNG FRUITS : Since the young developing fruits are the most vulnerable and susceptible to rotting and result in serious losses, the factors affecting their rotting were investigated.

Temperature : Healthy young fruits of the size of 8-10 × 4.6 cm. were used for artificial inoculation tests at different temperatures. All the floral parts were removed just before inoculation and their surface sterilized. Two to three drops of spore suspension of the fungus containing about 1×10^3 spores/ml. were put at the distal end of the fruit. These inoculated fruits were held in upright position in small moist chambers and placed at different temperatures. Ten young fruits were used for each test. The daily advance of rotting of the fruits as indicated by their browning and rotting was measured from the distal end and the results expressed in Table 3.

The optimum temperature for rotting of young fruits by the pathogen was found to lie in the range of 28-32°C. Rotting of the fruits proceded rather slowly at 16° and 36°C. It took 3 days to completely invade the fruit at 28°C ; 4 days at 32°C and 8 days at 16° and 36°C. The conidial production was profuse on the rotted fruits kept at 24-32°C.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Rotting of young Pumpkin fruits at different temperatures and relative humidities when inoculated with *Cunninghamella echinulata*.

Temperature °C	Average daily advance of rotting (mm.)	Relative humidity per cent	Average daily advance of rotting (mm.)
16	9	0	0
20	17	20	8
24	27	32	14
28	30	55	19
32	28	65	21
36	11	88	28
40	0	100	30

Relative Humidity : In the field the progress of rotting of fruits was found to be affected by the atmospheric humidity. In the laboratory, experiments were designed to study the effect of different relative humidities (RH) on the progress of the fruit rot caused by the fungus. Different RH were maintained in 8 oz. wide mouthed bottles containing solutions of different salts, as suggested by Clayton (1942) to maintain different RH. The young fruits were inoculated by the method described earlier, and inoculated fruits kept at 28°C for incubation. Advance of rotting was observed periodically and the results are given in Table 3.

The advance of rotting was best seen at 88-100 per cent RH, though at 55 per cent RH complete rotting of the fruit was seen after 6 days. At 32 per cent RH or below the rot proceded slowly and no conidial heads were seen.

CONTROL OF DISEASE BY FUNGICIDAL SPRAYS : To control the disease in the field, some fungicidal trials were made. For this each of the 3 plots were divided into 5 equal parts, and each such part was sprayed with fungicides. Thus each fungicidal treatment was replicated thrice and four fungicides were sprayed in each test site, keeping the fifth one as control. The spraying operations were begun when the plants were about 4-5 weeks old and had initiated the daily rythum of opening and closing of flowers. In one of the plots the fungicidal sprayings were given after every ten days till harvest ; in the second plot the plants were sprayed only twice ; *i. e.* once at the flowering stage and the second after 3 weeks of first spraying ; while the third plot received only one spraying in the beginning. The evaluation of different spray schedules and different fungicides was made by counting the total number of flowers at the time of spraying and comparing them with the controls. The fungicides used were : Actidione (Cycloheximide ; pure, Beta-[2-(3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide) ; Phaltan 50 wettable

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(n-trichloromethyl thiophthalimide) ; Phygon XL (Diclone, 2, 3-dichloro-1, 4-naphthoquinone) ; and Bordeaux mixture. The results are given in Table 4.

TABLE 4 : Comparison of four fungicidal sprays to control pumkin rot.

Fungicide	Dosage	No. of spraying	Per cent flowers infected	Remarks
Actidione	2ppm	1	2.5	Flowers become infected after 3-4 weeks
		2	1.0	—Do—
		4	0	Phytotoxicity of leaves
Phaltan	1½ lb/100 gal.	1	6.5	
		2	1.5	
		4	0	No phytotoxicity
Phygon XL	1 lb/100 gal.	1	18.5	Plants become infected after 3-4 weeks
		2	7.0	
		4	2.0	
Bordeaux mixture	4 : 4 : 100	1	5.0	
		2	3.5	
		4	1.0	Slight bronzing of leaves.
Control			68.0	

It was seen that 4 sprays of Actidione or Phaltan completely checked the disease, though there was some phytotoxicity with Actidione, which was absent in case of Phaltan. Only one spray with any fungicide did not check the disease effectively, since infection in flowers and young fruits reappeared after 3-4 weeks of first spraying due to the development of new flowers. Although higher concentrations of Bordeaux mixture were not tried, yet it appeared that it would prove phytotoxic, since after 4 sprayings there was slight bronzing of leaves. Phygon, however, was not satisfactory in checking the disease.

DISCUSSION AND SUMMARY

Cunninghamella rot of Spanish pumkin manifests mainly on young fruits, besides affecting flowers, leaves and petioles, resulting in severe

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

losses in the yield of the crop. The young fruits and opened flowers rotted earlier than other parts of the plant body. Although stem could be artificially infected, yet it has not been found diseased in the field.

The pathogen proliferates readily on variable kinds of substrates. Maximum growth of the fungus was obtained at a temperature of 28-32°C at pH 6.0-6.5. For spore germination the optimum temperature was 24-32°C at pH 6.5 and 100 per cent relative humidity. The infection of young fruits, on the other hand, was best at 28-32°C and 88-100 per cent relative humidity. Moisture and temperature apparently seemed critical in the proper development of the disease, for in the field it was observed that the advance of rotting of plant tissues was arrested with the increase or decrease in temperature or decrease in relative humidity.

From the etiological study of the disease it was observed that the pathogen overwinters in dried-up mummies (also perhaps dead stubble) and in the soil. The dead mummies appeared to be primary source of infection since these harboured viable fungus for a long time (32 months or more) in the dead tissues which readily produced abundant conidia in the presence of moisture. Besides, the fungus survived deep in the soil for most part of the year, and the incidence of high inoculum potential was greatest when the flowers began to open. It is probable that crop rotation may eliminate the fungal population of the soil. Conidia, however, seemed to be more virulent in infecting plant parts than the vegetative mycelium.

The disease could be effectively controlled by spraying four times with Phaltan or Actidione, and less so by Bordeaux mixture or Phygon. One or two sprayings with any of these fungicides gave partial control, since with the development of new flowers fresh infections occured and fresh sprayings were, therefore, necessary for their protection. Actidione though satisfactory in controlling the disease, yet proved to be phytotoxic on pumkin plants at the concentrations sprayed. The effectiveness of Phaltan may also be explained on the basis that it has been found to be good in checking the pectolytic enzyme activity of the fungi, which is responsible for this disease in pumkin (Grover, 1964 ; Grover and Moore, 1962).

ACKNOWLEDGEMENTS : Sincere thanks are due to Dr. C. W. Hesseltine for confirming the identity of the organism and his comments about it. Thanks are also due to farm-owners for their co-operation in the field experiments and to Prof. P. N. Mehra for facilitating these investigations.

Department of Botany,
Punjab University,
Chandigarh 3, Pb.

REFERENCES

CLAYTON, C. N. (1942) The germination of fungus spores in relation to controlled humidity. *Phytopathology* **32** : 921-943.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FITZPATIRCK, H. M. (1930) *The Lower Fungi. Phycomycetes.* McGraw Hill, N. York.

GILMAN, J. C. (1957) *A manual of Soil Fungi.* 2nd. Ed. Iowa State College Press, Ames, Iowa, USA.

GROVER, R. K. (1964) Effect of some fungicides on pectolytic enzyme activity of *Sclerotinia sclerotiorum* and *Botrytis allii. Phytopathology* **54** : 130-133.

GROVER, R. K. AND J. D. MOORE (1962) Toximetric studies of fungicides against brown rot organisms, *Sclerotinia fructicola* and *S. laxa. Phytopathology* **52** : 876-880.

HESSELTINE, C. W. (1955) Genera of Mucorales with notes on their synonymy. *Mycologia* **47** : 344-363.

LENDER, A. (1908) *Les Mucorinees de la Suisse.* 180 p.

NAUMOV, N. A. (1939) *Cles des Mucorinees.* Paul Lechevalier, Paris.

*NEKLYUDOVA, MME E. T. (1960) (Diseases of pumpkin. In Russian) *Zasch. Rast. Moskva*, **5** : 55-56.

WARCUP, J. H. (1951) The soil plate method for isolation of fungi from the soil. *Nature*, London, **166** : 117.

ZYCHA, H. (1935) Mucorineae. In Kryptogamenflora d. Mark Brandenburg, **6a** : 1-264.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON PUCCINIA HORDEI I. PHYSIOLOGIC SPECIALIZATION AND SCREENING OF VARIETIES

L. M. JOSHI, R. PRASADA AND L. B. GOEL

(Accepted for publication July 29, 1965)

Since Stakman and Piemeisel (1917) demonstrated that stem rust of wheat (*Puccinia graminis* var. *tritici* (Pers.) Erikss. and Henn.) consists of a number of physiologic races differing mainly in pathogenicity, considerable amount of literature has accumulated on the occurrence, distribution and prevalence of races of cereal rusts. In *Puccinia hordei* Otth, Mains (1926) first demonstrated the presence of two races in the United States. Since then such studies have been carried out by Waterhouse (1929), Luig and Baker (1956) in Australia ; Brown and Newton (1929) in Canada ; Hey (1931), Ronsdrop (1934, 1935), Straib (1937), Nover and Mansfield (1959) in Germany ; Hirschhorn (1933), Vallega *et al.* (1954) and Cenozk (1961) in Argentina ; D'Oliveira (1939) in Portugal ; Levine and Cherewick (1952), Moseman and Roane (1959) in U. S. A.; da Silva (1959) in Brazil ; Boasso (1957) in Uruguay; Joshi *et al*, (1959) and Vasudeva, Joshi and Sreekantiah (1960) in India and Hermansen (1962) in Nordic countries. A consolidated account of the occurence of races in different parts of the world and a key for identification of 52 races reported by various workers was given by Levine and Cherewick (1952).

MATERIAL AND METHOD : For our studies, differential varieties selected by Levine and Cherewick (1952) were used. Seedlings were raised in 4″ pots under spore-proof conditions of the greenhouse and were always inoculated when the first leaf had fully unfolded and the second leaf was just emerging out. Inoculations were done either with lancet needle or with a mixture of uredo-talc (1 : 10 ratio) by blowing it on seedlings thoroughly sprayed with water before hand. The plants were kept in moist chambers in 100 per cent humidity for 20-24 hours.

EXPERIMENTAL RESULTS : In all 59 samples were analysed and information regarding races met with is set out in Table 1.

TABLE 1 : Number of samples analysed and races met with from different states during 1957-1963.

Name of states	Crop years					
	1956-57	1957-58	1958-59	1959-60	1960-61	1961-62
Delhi	(3)H_1, H_2	(7)H_1	(4)H_1,H_2	(14)H_1,H_2	(6)H_1, H_2,H_3	(3)H_2, H_3
Uttar Pradesh	—	(1)H_2	(1)H_1	(3)H_1,H_2	(1)H_1	—
Bihar	(4)H_1, H_2	—	(8)H_1, H_2	—	—	—
Madras	(1)H_1, H_2	(1)H	—	(1)H_2	(1)H_2	—

No. of samples analysed is given in brackets.

Races H_1, H_2 and H_1-A have been reported earlier and race H_3 is a new race.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Reactions of differential varieties* to all 3 Indian races of *Puccinia hordei*

	Speciale	Reka**	Sudan	Bolivia	Oderbrucker	Quinn.	Egypt-4	Gold	Latchlaler	Cruzat	Chilean-D	Club Mariout	Berg	Samaria	Austral	Kinver	Local
H_1	3-4	0 ; -1	4	0 ; -1	4	0	3-4	0	0	4	3-4	3-4	4	4	0 ; -1	3-4	4
H_2	4	0 ; -1	4	0 ; -1	4	0	3-4	0	0	3-4	4	3-4	4	3-4	3-4	4	4
H_3 (New Race)	3-4	0 ; -2	4	0 ; -2	4	0	3-4	0	0	3-4	0 ;-2	0 ; - 2	3-4	3-4	0-1	0 ; -1	4

*Even though this differential shows mostly resistant type of reaction to all the 3 known races, occasionally it shows suceptible type of reactions also.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The reaction of race H_3 which was first isolated from Delhi from 1960-61 Crop, are set out in Table 2 alongwith those of earlier recorded races.

The Key compiled by Levine and Cherewick (1952) summarises the reactions of only 19 critical differentials in the consolidated table. Perhaps, they themselves did not consider the non-critical differentials as important ones. In practice too, some workers (Moseman and Roane, 1956 ; da Silva, 1959) are not using the non-critical differentials. This arrangement, however, has not been found suitable for Indian collections. When the work was first started in India (Joshi *et al.*, 1959) in absence of recognized differential varieties, two races were identified on the basis of their reactions to two Afghan selections but these races could not be separated on any of the 16 differentials. Even the 3 races, described above (*viz.*, H_1, H_2 and H_3) do not show any differential response on any "critical" differentials and their differences are only on non-critical ones, namely on Austral, Chilean-D, Club-Mariout and Kinver. However, our experience is based on the study of a limited number of samples and, therefore, a definite conclusion as to the usefulness of these differentials cannot be drawn at present. It is, suggested that for a few more years, the complete set of 16 differentials along wtih two or three others varieties (like Afghan selections) may be used to assess properly the usefulness limitations of the present set of differentials. In the past for similar considerations, Hey (1931) and Ronsdorf (1935) felt that Mains's set (1931) was not suitable for European races.

SCREENING OF VARIETIES : A. *Seedling tests* ; For screening, the seedlings were raised in 4″ pots under spore-proof glasshouse conditions and inoculated, either with individual races or a mixture thereof. The recording of reactions and use of symbols to designate resistance or susceptibility of a particular variety was done in the same way as for identification of races (Levine and Cherewick, 1952). Observations were recorded 10 to 12 days later.

Results of seedling resistance tests are given below :—

I. MIXTURE OF RACES : 1. Mixture of races H_1, H_2 and H_1-A.

(a) The following varieties were found to be resistant :—
E. B. Nos. 438 and 527.

(b) The following varieties were found to be susceptible :—
C. 138-2, C. 251, NP. 13, NP. 21, NP. 103, NP. 104, B. R. 22 and R. S. 17.

(c) The following variety showed mixed reactions :—
E. B. 525.

2. Mixture of races H_1, H_2, H_3 and H_1-A :

(a) The following varieties were found to be resistant :—
E. B. Nos. 78, 675, 1109, 1170, 1195, 1203, 1205, 1207, 1208, 1213, 1373, 1398, 1413, 1422 and 1434.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(b) The following varieties were found to be susceptible :—

E. B. Nos. 680, 934, 1026, 1121, 1124, 1132, 1133, 1135, 1137, 1143, 1145, 1147, 1150, 1151, 1157, 1160, 1172, 1173, 1174, 1193, 1196, 1201, 1204, 1206, 1209, 1210, 1224, 1228, 1238, 1240, 1269, 1270, 1274, 1284, 1374, 1386, 1389, 1390, 1392, 1395, 1396, 1400, 1402, 1403, 1404, 1421, 1423, 1428, 1431, 1433, 1436, 1437, 1439, 1441, 1445, 1447, 1457, 1458, 1459, 1463, 1466, 1468, 1477, 1479, 1480, 1481, 1482, 1485, 1503, 1504, 1514, 1518, 1519 and 1578.

(c) The following varieties showed mixed reactions :—

E. B. Nos. 1175, 1176, 1199, 1202, 1306, 1420, 1486, 1510, 1516, 1517.

II. INDIVIDUAL RACES : 1. (a) The following varieties were susceptible to races H_1 and H_2 :—

E. B. Nos. 200, 240, 387, 483, 488, 517, 518, 520, 521, 523, 546, 550, 571, 579, 583, 602, 606, 614, 615, 618, 620, 625, 627, 631, 633, 637, 639, 643, 644, 645, 661, 662, 666, 667, 685, 686, 673, 678, 682, 683, 687, 705, 711, 712, 713, 714, 715, 718, 720, 721, 735, 751, 753, 754, 755, I. B. 35, I. B. 74 and N. D. 12.

(b) The following variety was resistant to races H_1 and H_2 :—
E. B. 1413.

2. (a) The following varieties were susceptible to races H_1, H_2 and H_1-A :—

E. B. Nos. 187, 700, 701, 846, 934, 939, 947, 961, 964, 1004 and 1026.

(b) The following varieties were resistant to races H_1, H_2 and H_1-A :—
E. B. Nos. 525, 527.

3. (a) The following varieties were susceptible to races H_1, H_2 and H_3 :—

E. B. Nos. 846, 1001 and 1005.

(b) The following varieties were resistant to races H_1, H_2 and H_3 :—
E. B. Nos. 1109, 1195, 1218, 1369 and 1373.

4. (a) No variety was found to be susceptible to all the four races (viz. H_1, H_2, H_3 and H_1-A).

(b) The following varieties were resistant to races H_1, H_2, H_3 and H_1-A :—

E. B. Nos. 156, 301, 323, 491, 496, 524, 798, 873 and 884.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

5. Varieties showed differential response when tested against two or more races individually

Name of Variety	Race H_1	Race H_2	Race H_3	Race H_1-A
E. B. 57	4	—	—	0 ;
E. B. 78	2-3	0 ;-1	0 ;	—
E. B. 153	4	3	—	0 ;
E. B. 190	x	0 ;-2	0 ;	0 ;
E. B. 195	4	3	—	0 ;
E. B. 196	4	0 ;-2	0 ;-1	0 ;-1
E. B. 201	x	x	—	x
E. B. 206	4	4	—	0 ;-1
E. B. 217	4	3	—	0 ;
E. B. 234	4	0 ;	—	0 ;
E. B. 300	4	x	—	0 ;-1
E. B. 526	3	3	—	0
E. B. 626	3-4	3	—	0 ;-1
E. B. 630	x	3	—	0 ;-1
E. B. 660	0 ;	3	—	0 ;-1
E. B. 675	3	0 ;	—	—
E. B. 717	4	3	—	0 ;
E. B. 724	3	4	—	0 ;
E. B. 744	4	3-4	0 ;-1	0 ;
E. B. 808	4	3	—	0 ;-1
E. B. 842	x	0 ;-1	—	0 ;-1
E. B. 913	3	x	—	0 ;
E. B. 932	3-4	3	—	0 ;-1
E. B. 947	4	4	0 ;-2	—
E. B. 954	3	2	—	0 ;
E. B. 955	3	3	—	0 ;-1
E. B. 959	4	4	—	0 ;-1
E. B. 971	4	2	—	3
E. B. 1001	x	0 ;-2	—	0 ;-1
E. B. 1006	3	3	0 ;-1	0
E. B. 1007	4	3	0 ;-2	3
E. B. 1018	4	3	—	0 ;-1
E. B. 1022	4	4	x	3
E. B. 1023	4	0 ;-1	0 ;-3	—
E. B. 1033	4	4	—	1
E. B. 1035	3-4	3	—	2
E. B. 1398	4	0	0	—
E. B. 1418	4	0 ;	0 ;	—

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

B. *Adult resistance tests* : A large number of varieties were tested in the adult stage for their resistance to leaf rust of barley under conditions of artificial infection. These varieties were sown in November and harvested in April. Susceptible barley varieties were used as infector varieties and sown all round the experimental plots. The plots were artificially inoculated with the method followed in case of wheat (Pal, Vasudeva and Kohli, 1954). Inoculations were done from February onwards. For recording the intensity of rust, Cobb's Scale modified by Malcher's and Parker was used. Along with rust intensity the type of reaction was also noted and symbols R. (Resistant) S (Susceptible) and SR. (Mixed S. and R types) have been used to denote different classes of rust reactions.

Varieties E. B. 156, E. B. 301, E. B. 323, E. B. 491, E. B. 496, E. B. 798 and E. B. 884 have shown complete freedom from rust for 3 years in succession in the adult stage and were also resistant, in the seedling stage, to all the known Indian races of *P. hordei*.

ACKNOWLEDGEMENT : Our thanks are due to Sri K. B. L. Jain, Division of Botany, Indian Agricultural Research Institute, for giving us necessary information about the name of variteies and their field reactions.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

REFERENCES

BOASSO, CELIA (1957) Physiologic races of some of the cereal rusts isolated in Uruguay in 1956. *Robigo* **4** : 11-12.

BROWN, A. M. AND M. NEWTON (1929) The dwarf leaf rust of barley in western Canada. *Canada Expt. Farms, Div. Bot., Ann. Rept.*, 1928 : 83-84.

CENOZ, H. P. (1961) The Existence of another physiologic race of barley leaf rust (*Puccinia hordei* Otth) in Argentina. *Robigo* **12** : 12-14.

DA SÍLVA, ADY RAUL (1959) Differential varieties used at the "Instituto Agronomico do sul" for rust race Identifications *Robigo* **7** : 6-7.

D' OLIVEIRA, B. (1939) Studies on *Puccinia anomala* Rostr. I Physiologic races on cultivated barleys. *Ann. Appl. Biol.* **26** : 56-82.

HERMENSEN, J. E. (1962) Physiologic Races of *Puccinia graminis* Var. *tritici*, *Puccinia recondita* and *Puccinia hordei* in Denmark and Sweden in 1960. *Robigo* **13** : 13-14.

HEY, A. (1931) Beitrage Zur Spezialisierung Des Gerstenzwergrostes, *Puccinia simplex* Erikss. et. Henn. *Biol. Reichsanst. f. Land U. Forstw. Arb.* **19** : 227-261.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

*HIRSCHHORN, J. (1933) Dos royas de al Cebada, nuevas para la Argentina. *Rev. Fac, Agron. Univ. Nac. La Plata* **19** : 390-397.

JOSHI, L. M., D. P. MISRA, K. R. SREEKANTIAH, V. C. LELE AND D. KAK (1959) Studies in *Puccinia hordei* Otth, the leaf rust of barley in India. *Indian Phytopath.* **12** : 69-75.

LEVINE, M. N. AND W. J. CHEREWICK (1952) Studies on dwarf leaf rust of barley. *U. S. D. A. Tech. Bull.* No. *1056.*

LING, N. N. AND E. P. BAKER (1956) A note on uredospore colour mutant in barley leaf rust (*Puccinia hordei* Otth). *Proc. Linn. Soc. N. S. W.* **81** : 115-118.

MAINS, E. B. (1926) Studies in rust resistance. *J. Hered.* **17** : 312-325.

————(1930) Host specialization of barley leaf rust, *Puccinia anomala. Phytopathology* **20** : 873-882.

MOSEMAN, J. G. AND C. W. ROANE (1959) Physiologic races of barley leaf rust (*Puccinia hordei*) isolated in the United States from 1956 through 1958. *Plant Dis. Reptr.* **43** : 1000-1003.

*NOVER, ILSE AND R. MANSFELD (1959) Resistenzeigenschaften in Gerstenund Weizensortiment Gatersleben. 3. Prufung der Gerten aut ihr Verhalten gegen Zwergrost (*Puccinia hordei* Otth). Kulturpflanze. (*Ber. Inst. Kulturpfl Forsch.*) **7** : 29-36.

PAL, B. P., R. S. VASUDEVA AND S. P. KOHLI (1954) *Breeding Rust Resistant Hill Wheats in India.* I. C. A. R. Research Series No. **5** : 52 pp.

RONSDORF, L. (1934) Einige Versuche uber Biologische Rassen des Gerstenzwergrostes. *Arb. Biol. Abt.* (*Anst.-Reichsanst*), *Bere.* **21** : 109-114.

————(1935) Weitere Untersuchungen uber den Nachweis Biologischer Rassen des Gerstenzwangrostes, *Puccinia simplex* Erikss Henn. *Phytopath. Z* **8** : 237-243.

STRAIB, W. (1937) Die Bestimmug Der Physiologischen Rassen des Gerstenz-Wergrostes, *Puccinia simplex* (Kcke.) Erikss. et Henn. *Biol. Reichsanst. f. Land U. Forstw.* Arb. **22** : 43-63.

STAKMAN, E. C. AND F. J. PIEMEISEL (1917) Biologic forms of *Puccinia graminis* on cereals and grasses. *J. Agric. Resh.* **10** : 429-95.

*VALLEGA, J., H. P. CENOZ, J. L. TESSI AND J. H. FRECHA (1954) Importancia de las enfermedades de los Cereals en 1953 Y Comportamiento de las Variedades en el gran Cultivo yen ensayos de resistencia. *Hoja Inst. Fitotecn., B. Aires* **15** : 24-1954.

VASUDEVA, R. S., L. M. JOSHI AND K. R. SREEKANTIAH (1960) Physiologic specialization in *Puccinia hordei. Indian Phytopath.* **13** : 66-67.

WATERHOUSE, W. L. (1929) Australian rust studies I. *Proc. Linn. Soc. N. S. W.* **54** : 615-680.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECT OF HEXOSE PHOSPHATES AND ULTRAVIOLET IRRADIATION ON PERITHECIAL DEVELOPMENT IN GLOMERELLA TUCUMANENSIS

A. K. DUTTA*, B. L. CHONA AND B. S. BAJAJ**

(Accepted for publication July 29, 1965)

The occurence of ascigerous stage (*Glomerella tucumanensis* (Speg) Arx and Mueller) of *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane, was first reported from Louisiana, U. S. A. by Carvajal and Edgerton (1943, 1944) and subsequently from Taiwan, Formosa (Ling and Ma, 1950) and India (Chona and Bajaj, 1953). Perithecia have been readily produced in Laboratory by inoculating dry, old sugarcane leaves with conidia of red rot fungus and incubating under high humid conditions (Chona and Srivastava, 1952), The perithecia rarely developed on green autoclaved leaves and failed to develop on medium devoid of intact plant parts. The perithecial development on the old, dry autoclaved leaves enriched by the addition of carbon and nitrogen sources was rather depressed (Ling and Ma, 1950 ; Chona *et al.* 1960). Isolates of *C. falcatum* showed considerable variability in capacity to produce perithecia and likewise, different varieties of sugarcane showed differences in their ability to bear perithecia on the dry, old leaves. Most of the isolates have been found to degenerate progressively in respect of sexuality when maintained on artificial culture media (Chona *et al.* 1961).

Studies made so far on the development of perithecia of red rot fungus suggest that probably a delicate nutritional balance exists in the dry old leaves for the development of perithecial stage of the red rot fungus. In order to gain further knowledge of the conditions favouring development of perithecial stage, studies on some of the factors, like addition of hexose phosphate and calcium and ultraviolet irradiation affecting perithecial development in the red rot fungus were made and results reported here.

MATERIAL AND METHODS : A highly-fruiting isolate of *C. falcatum* obtained from sugarcane mid-rib lesions was used in these studies. Dry and straw-coloured old cane leaves and mature and healthy green leaves of variety, Co. 312, free from mould and other blemishes were collected and pieces, measuring 6 cm. in length, were taken from the middle of the leaves. The old leaf-pieces were soaked in distilled water for one hour and then placed on a thin pad of moistened cotton wool in a Petri plate. To the green leaf pieces, instead of pre-soaking in water, a washing with distilled water was given. The plates containing leaf-pieces were sterilised at 15 lb. p. s. i. for 20 minutes unless otherwise stated. A spore

---

The paper is based on part of thesis submitted by Senior author for Ph.D. degree of Indian Agricultural Research Institute, New Delhi.

**Present address* : Ranchi Agricultural College, Kanke, Bihar.

**Present Address—Deputy Supdtt. Antibiotics project, Virbhadra, Rishkesh (U.P.)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

suspension having 30-40 conidia per microscopic field (L. P.) was prepared from 20-day old cultures and a drop of this placed at the centre of each side of the leaf blade and spread out uniformly by means of a sterile loop. The inoculated leaf pieces were incubated at a25°C for 10 days and, thereafter, the number of perithecia was counted by mens of a colony counter and a magnifying lens from 5 different spots of known area selected at random from each leaf, and the intensity expressed as number of perithecia per sq. cm. Three plates, each having two leaf pieces. were kept in each treatment. The intensity of mycelial growth, acervulation and perithecial development was observed visually also and designated as + + + + + for profuse, + + + + for abundant, + + + moderate + + for scanty and + for extremely poor development.

EXPERIMENTAL : *Effect of hexose phosphates and calcium on perithecial formation* : The effects of hexose phosphates and calcium salt were studied by soaking the pieces of uniform size of (i) old dry cane leaves, (ii) old dry leaves previously boiled in distilled water for half an hour and (iii) green sugarcane leaves in equal quantities of 0.025 per cent (W/V) solution of glucose-6-phosphate (barium salt), 0.025 per cent (W/V) solution of fructose—1 : 6 diphosphate (dibarium salt) and 0.5 % (W/V) solution of calcium chloride for 15 hours in Petri dishes on cotton-wool pads. The plates with leaf pieces and respective solution were sterilised at 10 lb. p. s. i. for 10 minutes and inoculated with the highly fruiting isolate. The observations in respect of mycelial growth, acervulation and perithecial production are set forth in Table 1.

TABLE 1 : Effect of hexose phosphates and calcium salt on perithecial formation of *Glomerella tucumanensis*.

Substrate	Amount of mycelium	Intensity of Acervulation	Av. No. perithecia per cm^2
Old, dry leaf + distilled water only (Control)	+	+	16.40
Old, dry leaf + hexose phosphates	+	+	28.63
Old dry leaf + hexose phosphates + calcium chloride	+	+	30.13
Boiled old, dry leaf	+ to ++	+	11.20
,, ,, ,, ,, + hexose phosphates	+ to ++	+	35.20
,, ,, ,, ,, + hexose phosphates + Ca Cl_2	+ to ++	+	22.83
Green leaves + dist. water	+++	+++	Nil
,, ,, + hexose phosphates	+++	+++	Nil
,, ,, + hexose phosphates + $CaCl_2$	+++	+++	Nil

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The addition of mixture of hexose phosphates resulted in a significant increase in the number of perithecia on old autoclaved leaves both with and without previous boiling in water ; the response being more pronounced in the case of former (Fig. 1). Addition of calcium chloride to hexose phosphates showed depressive effect on perithecial formation on the leaves where nutrients were depleted by boiling. Green leaves, which were rich in available nutrients, however, showed greater mycelial growth and acervulation but no perithecial formation in any of the treatments.

Because of stimulatory response shown by hexose phosphates in inducing perithecial formation and inhibitory response by calcium, an attempt was made to analyse the extracts of dry, old and green leaves of sugarcane chromatographically for the presence or absence of hexose phosphates and calcium.

To prepare leaf extract, 5 g. of clean pieces of dry and green leaves were boiled for about 30 minutes with 100 ml. distilled water and the extract was squeezed out through cheese cloth and evaporated to 50 ml. in water bath and filtered to remove the scum. The solvent system—methanol/formic acid/water (80-15-5) (Bandurski and Axelrod, 1951) and the spray reagent of a mixture of ammonium molybdate, perchloric acid, hydrochloric acid and water (Hanes and Ishorwood, 1949) were employed for chromatographic analysis (ascending) of glucose-1-phosphate, glucose-6-phosphate and fructose-1 : 6-diphosphate whereas the method described by Pollard *et al.* (1951) was followed for the detection of calcium. Equal volume of solutions of the above chemicals in pure form were deposited simultaneously for comparison on whatman filter paper No. 1. For the detection of the glucose-1-phosphate, the paper, after spraying, was dried at 60°C for 5 minutes and for glucose-6-phosphate and fructose-1 : 6-diphosphate it was further autoclaved for 2 minutes at 10 lb. p. s. i. and exposed to ammonia vapours.

The Rf values of the unknown spots and those of the known compounds chromatographed simultaneously revealed the presence of glucose-1-phosphate, glucose-6-phosphate, fructose-1 : 6-diphosphate and calcium in the leaf extract of both the old, dry and green leaves. This shows that it is not mere presence or absence of hexose phosphates which influence the perithecial production on sugarcane leaves but probably their combination with low nutrition favours the formation of ascigerous stages in this organism.

To confirm this, an experiment was set up with green and dry leaves which were boiled with 70 per cent alcohol for one hour in a water bath and subsequently washed thrice with hot distilled water. Green leaf pieces after surface sterilisation with 0.1 per cent mercuric chloride solution, were also included in the experiment. The leaves were inoculated with the conidial suspension of the red rot fungus and incubated in Petri dishes containing distilled water, solution of hexose phosphates or leaf extract of old, dry leaves. The observations on the growth, acervulation and perithecial formation were recorded visually and are presented in Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

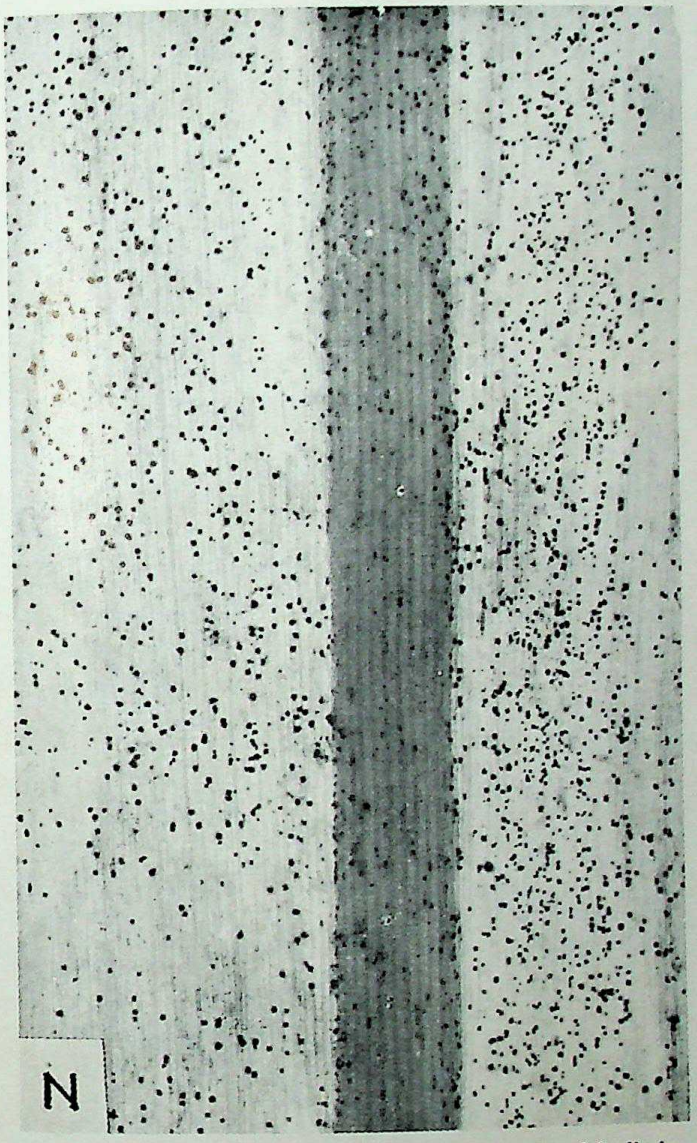


Fig. 1. N Perithecia of *C. falcatum* on old and boiled, autoclaved leaves impregnated with a 0.05 per cent solution of mixed hexose phosphates.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Effect of addition of hexose phosphates and cane leaf extract to green and dry leaves of sugarcane on perithecial development of *G. tucumanensts*

Treatment	Intensity		
	Growth	Acervuli	Perithecial
Green autoclaved leaves	+++	+++	Nil
Green alcohol extracted leaves	+++	++	+
Green alcohol extracted leaves plus hexose phosphates	+++	+++	++
Green alcohol extracted leaves plus leaf extract	++	+++	++
Green surface sterilised leaves	++	+	++
Green surface sterilised leaves plus hexose phosphates	+++	++	Nil
Green surface sterilised leaves plus leaf extract	+++	++	Nil
Dry, old autoclaved leaves	++	++	++++
Dry, old alcohol extracted leaves	+	+	++
Dry, old ,, ,, ,, + hexose phosphates	++	++	++++
Dry, old alcohol extracted leaves + leaf extract	++	++	+++

Having seen the stimulatory effect of the hexose phosphate on perithecial development on old, dry leaves and green alcohol extracted leaves, an attempt was made to examine their effect on perithecial formation on culture medium. The synthetic medium of Asthana and Hawker (1936) with some modification* and cane leaf extract agar medium** as semi-synthetic medium were employed as basal media and supplements like, glucose-6-phosphate, fructose-1 : 6-diphosphate and calcium chloride were incorporated separately as well as in mixture in amounts to give concentration of 0.05 per cent of the esters and 0.5 per cent of calcium. Addition of the hexose phosphates was made by the method described by Hawker (1948). Treatments without the organic phosphates and calcium were kept as control. Unlike the results obtained on the dry and alcohol extracted green leaves, perithecia did not develop in the culture medium

*The amounts of glucose was reduced, only 2.0 g. per litre of water was used.

**12.5 g. of old, dry leaves of cane variety Co. 312 were boiled in 250 ml. of distilled water for an hour over a low flame. The clear extract was concentrated to about 125 ml. by heating in a water bath. After filtering agar was added at the rate of 2 per cent of the extract and the medium was sterilised at 10 lb. p. s. i.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

containing hexose phosphates and no noticeable effect of these substances could be found on the vegetative or asexual stages of the fungus.

Effect of ultra-violet irradiation on perithecial formation : Effect of UV irradiation on perithecial formation in the case of the highly fruiting and a sparsely fruiting isolate of *C. falcatum* was studied. The young and actively growing mycelium of the isolates on old, dry autoclaved cane leaves was irradiated with U. V. light at 2537°A from a Hanovia chromatolite lamp of 30 watts fitted with Wood's filter. The plate cultures were placed, open, at a distance of 12.7 cm. from the lamp under aseptic conditions. The observations were recorded as usual after 10 days. The results (Fig. 2) showed that ultraviolet light adversely affected perithecial production in the highly fruiting isolate whereas in the case of the sparsely fruiting isolate the perithecial production remained unaffected. In the latter case the mycelial growth increased with the increase in dosages up to 40-minute exposure and, thereafter, it showed depressing tendency whereas in the former case the mycelial growth increased between 40 and 60-minute irradiation and thereafter, it decreased.

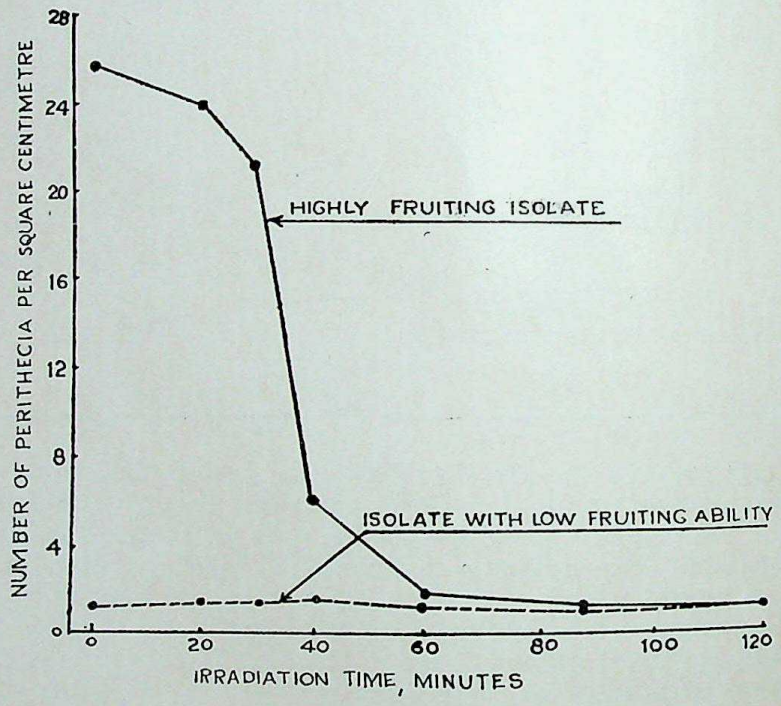


Fig. 2. Effect of ultraviolet radiation on the production of perithecia by isolates of *C. fal catum.*

DISCUSSION : *Glomerella tucumanensis* does not form its perfect stage on culture media but perithecia of fungus are abundantly produced on dry, old sugarcane leaves in nature and under artificial conditions in the laboratory (Carvajal and Edgerton, 1943 ; Chona and Srivastava, 1952 ; Chona and Bajaj, 1953). On green autoclaved sugarcane leaves perithecia of fungus are rarely formed unless certain treatments are given to the green leaves which may result in the production of few smaller sized perithecia. In the present investigations it has been observed that

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

addition of small amounts of mixture of hexose phosphates increased significantly the number of perithecia on dry, old autoclaved leaves but not on green autoclaved leaves. The latter are relatively richer in nutrition and are suitable for vegetative and asexual stages and addition of hexose phosphates had no effect on this substratum. Stimulation in the production of perithecia and asci of some other ascomycetes by addition of hexose phosphates to the substrate has been observed by Hawker (1947, 1948), Buston *et al.* (1953) and Miller *et al.* (1955).

Addition of hexose sugars, nitrogenous sources, and calcium to dry, old sugarcane leaves have been reported to depress perithecial production and stimulate mycelial growth in this fungus (Chona *et al.* 1961) but the hexose phosphates which have been known to accelerate the rate of respiration in fungi behaved differently and had very favourable effect on fruiting of this organism.

On old leaf media, the deleterious effect of calcium on perithecial production as reported by Chona *et al.* (1961) was counter balanced by addition of hexose phosphates while on boiled old leaves where most of nutrients were removed, calcium had an adverse effect on fruiting and addition of hexose phosphates could not bring any change on depressive effect of calcium on sporulation of *G. tucumanensis.*

The presence of the hexose monophosphates, fructose-1 : 6-diphosphate and calcium was detected chromatographically both in the green and dry, old leaf extracts. The scarcity of perithecia on the green autoclaved leaves or their abundanee on dry old autoclaved leaves do not therefore, appear to be correlated with the mere presence of the organic phosphates ; probably their combination with low nutrition favours the formation of the perfect stage in this fungus. This was confirmed by further experiments using substrates such as alcohol extracted green and dry, old sugarcane leaves. To these substrates with lower nutrient contents, the addition of hexose phosphates or extracts of old sugarcane leaves stimulated perithecial production a great deal. On the other hand, on surface sterilised green leaves which have lesser amount of available nutrients there was some perithecial production but this was found to go down with the addition of either the hexose phosphates or extracts of old sugarcane leaves. Ultraviolet light adversely affected the perithecial production in a highly fertile isolate of *G. tucumanensis*, and in a degenerated isolate, treatment with the light failed to improve the fruiting ability. Stimulation in perithecial formation with simultaneous decrease in mycelial growth by ultraviolet light treatment has been noted in *Glomerella cingulata* (Stevens, 1928), but the result obtained on *G. tucumanensis* was very different. These results partly explain the physiological conditions conducive for perithecial development in this fungus.

SUMMARY

The addition of small amounts of glucose-6-phosphate and fructose-1 : 6-diphosphate caused a significant increase in the intensity of perithecia on dry, old autoclaved leaves but there was no effect of the hexose phosphates in respect of sporulation of the fungus on culture medium.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The hexose monophosphates and fructose-1 : 6-diphosphate were detected in the extracts of green and dry, old sugarcane leaves. It was observed that the hexose phosphates in combination with low nutrition-content of the substrate favour the formation of perithecia of the fungus. Treatment with ultraviolet light adversely affected the perithecia forming ability of a highly fruiting isolate.

ACKNOWLEDGEMENT : Our grateful thanks are due to Dr. R. S. Vasudeva, former Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his keen interest and valuable suggestions in these studies.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

ASTHANA, R. P. AND L. E. HAWKER (1936) The influence of certain fungi on the sporulation of *Melanospora destruens* Shear and of some other Ascomycetes. *Ann. Bot.* **50** : 325-344.

BANDURSKI, R. S. AND B. AXELROD (1951) The chromatographic identification of some biologically important phosphate esters, *J. Biol. Chem.* **193** : 405.

BUSTON, H. W., A. JABBAR AND D. E. ETHERIDGE (1953) The influence of hexose phosphates, calcium and jute extracts on the formation of perithecia by *Chaetomium globosum J. gen. Microbiol.* **8** : 302-306.

CARVAJAL, F. AND C. W. EDGERTON (1943) The perfect stage of *Colletotrichum falcatum* (Abstract) *Phytopathology* **33** : 2.

CARVAJAL, F. AND C. W. EDGERTON (1944) The perfect stage of *Colletotrichum falcatum. Phytopathology* **34** : 206-213.

CHONA, B. L., B. S. BAJAJ, H. O. AGRAWAL AND T. N. SREENIVASAN (1961) A note on the factors influencing perithecial production in *Glomerella tucumanensis* (Speg) Arx and Mueller. *Proc. 4th All India Conference of Sugarcane Research and Development Workers, Waltair*-1960.

CHONA, B. L. AND B. S. BAJAJ (1953) Occurrence in nature of *Physalospora tucumanensis* Speg. the perfect stage of sugarcane Red Rot organism in India. *Indian Phytopath.* **6** : 63-65.

——————— AND D. N. SRIVASTAVA (1952) The perithecial stage of Colletotrichum falcatum Went in India. *Indian Phytopath.* **5** : 158-160.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

HANES, C. AND F. A. ISHERWOOD (1949) Separation of the phosphoric esters on the filter paper chromatogram. *Nature* **164** : 1107.

HAWKER, L. E. (1947) Further experiments on growth and fruiting of *Melanospora destruens* Shear in the presence of various carbohydrates, with special reference to the effects of glucose and sucrose. *Ann. Bot. N. S.* **11** : 245-260.

HAWKER, L. E. (1948) Stimulation of the formation of perithecia of *Melanospora destruens* Shear by small quantities of certain phosphoric esters of glucose and fructose. *Ann. Bot. N. S.* **12** : 77-79.

LING, K. C. AND P. T. MA (1950) A preliminary study on the perfect stage of the causal organism of red rot of sugarcane. *Proc. Congr. Int. Soc. Sugarcane Techs.* Hawai, 503.

MILLER, J. J., J. CALVIN AND J. H. TREMAINE (1955) A study of certain factors influencing sporulation of *Saccharomyces cerevisiae*, *Canad. J. Microbiol.* **1** : 560-573.

POLLARD, F. H., J. F. W. MCOMIE AND H. M. STEVENS (1951) The analysis of inorganic compounds by partition chromatography. *J. Chem. Soc.* part III, pp. 771.

STEVENS, F. L. (1928). The sexual stage of fungi induced by ultraviolet rays. *Science, N. S.* **68** : 514-515.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXTRACTABILITY OF NUCLEIC ACIDS FROM THE CALLUS TISSUES OF PHYLLOXERA GALL AND GRAPE STEM

H. C. ARYA

(Accepted for publication July 29, 1965)

A number of methods are in use for the estimation of nucleic acids in higher plants. The commonly used methods are : alkali extraction followed by acid hydrolysis (Schmidt and Thannhauser, 1945), cold and hot perchloric acid extraction (Ogur and Rosen, 1950) and hot extraction (Martin and Morton, 1956). The important role of nucleic acid contents of the plant tissues in the process of normal and diseased growth in plants has been emphasized by several authors (Robson, Budd and Yost, 1959 ; Woodstock and Skoog, 1960, 1962 ; Arya, Hildebrandt and Riker, 1962 and Arya, 1963, 1964). In the gall disease of bean induced by *Agrobacterium rubi* (Hildebrand) Starr. and Weiss., transformation of normal cells into diseased cells is accompanied by marked increase in their ribonucleoprotein content. Increased deoxyribonucleic acid (DNA) contents are in part associated with increased mitotic frequency (Klein, 1953 ; 1958 ; Klein, Rasch and Swift, 1953 ; Braun, 1954 ; Rasch, Swift, and Klein, 1959).

Plant tissue culture methods are very useful for examining the nucleic acid contents and their importance to cellular growth (Skoog, 1954). The first problem was to use a suitable method for the extraction of RNA and DNA out of the three methods given above or to modify any one of them to obtain maximum extractions. The second problem was the physical state of the tissues at the time of processing it for nucleic acid extraction. Differences between plant tissues prohibit any single formulation. Use of fresh tissue may be considered best for the purpose. However, when a large number of tissue samples are to be handled it is almost impossible to use fresh tissue. In such a case reliance on dry tissue (oven-dry or vaccum-freeze-dry) is a necessity.

A comparative study of the extractability of nucleic acids from the three types of the tissues (fresh, oven-dry and freeze-dry) was carried out. The results obtained are presented in this paper.

MATERIAL AND METHODS : Two single-cell clones, one each from normal grape stem (GS6-24), and the other from the gall tissue (GP4-21) incited by *Phylloxera vastatrix* Planch. were grown on modified White's basal mineral salt agar medium with 2.0 per cent sucrose, supplemented with 15.0 per cent coconut milk, alpha-naphthaleneacetic acid (NAA, 0.1 mg./litre), and calcium pantothenate (2.5 mg./litre). The medium was adjusted to pH 5.9-6.0 before autoclaving which remained unchanged afterwards.

Four seed tissue transplants, weighing approximately 35 mg., from three week old actively growing cultures, were grown in each of 6-oz

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

prescription bottles on 40 ml. of the medium. The cultures were incubated in the dark room at 26°±2°C and 55 per cent relative humidity.

The growing callus was taken out of the bottles and adhering agar removed by rolling it over the cheese cloth folds. Three samples from every clone, each weighing around 4.0 g. were taken and macerated in cold ethanol (90.0 per cent). This material was used as fresh tissue for further processing. For drying the tissue in a vacuum-freeze-drier, the samples weighing around 4.0 g. were macerated in cold ethanol and dried in the machine within 4-5 hrs. For drying the tissue in the oven at 65°C, the above amount of the tissue was first macerated in cold ethanol and then dried in the oven at 65°C. and stored in a desiccator.

The dried tissue was powdered and 250 mg. of each sample was suspended in 10 ml. of cold 70.0 per cent ethanol. In the case of fresh tissue, 4.0 g. of each sample was taken in 10 ml. of cold (70.0 per cent) ethanol. The nucleic acid fractions were obtained with the method of Ogur and Rosen (1950) as modified by Kupila *et al.* (1961) and Arya *et al.* (1962). Only a summary of the method is given here.

The samples were homogenized in Potter-Elvehjem homogenizer for 10 minutes and centrifuged at 4°C. This and subsequent centrifugation was done at 2,500 r.p.m. for 30 minutes. The residue was resuspended in 5 ml. of cold acidic ethanol (70.0 per cent ethanol with 0.1 per cent perchloric acid) and centrifuged at 4°C. Ethanol-ether soluble compounds were removed by treating the residue with 5 ml. of 3 : 1 ethanol and ethyl-ether. A few small glass beads were added and the mixture was boiled gently for 3 minutes on a water bath. The process was repeated, using the same volume of ethanol-ether. The residue was suspended in 5 ml. of cold 0.2 N perchloric acid ($HClO_4$) to remove acid soluble compounds and centrifuged at 4°C. The process was repeated and the steps completed in less than 2 hrs.

Samples of residue for RNA extractions were suspended in 5 ml. of 2.0 N concentration of HCl04 after removing acid soluble compounds by the above method. The samples were kept afterwards on a reciprocal shaker at 4°C for 18 hrs. with additional casual stirrings with a glass rod. The suspension was centrifuged and residue extracted three successive times with 5 ml. portions of cold 2.0 N concentrations of $HClO_4$. All the four extracts of each sample were combined to make 20 ml.

Samples of the residues from the above four extractions were suspended separately in 5 ml. of 0.5 N HCl04 for DNA extractions, and heated in a water-bath for 20 minutes at 74°C. and centrifuged. The process was repeated twice and the extracts were combined to make 15 ml. in each case.

RNA fractions were analysed on the Beckman 'DU' spectrophotometer in the ultraviolet range at 260 mμ. DNA fractions were analysed by the colorimetric method with diphenylamine. Diphenylamine (Reagent grade, Fisher Scientific) was dissolved in glacial acetic acid at a final concentration of 100 g./litre of solution and stored in a dark bottle

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

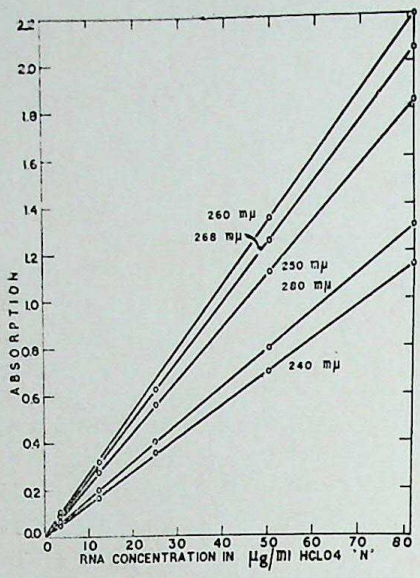


Fig. 1

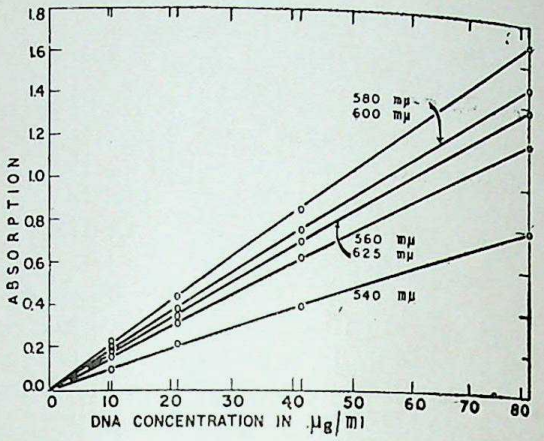


Fig. 2

Fig. 1—2. 1. Standard ribonucleic acid (RNA) curves at different wave lengths in the 'DU' spectrophotometer prepared with chemically pure RNA. Maximum spectrophotometric absorption took place at 260 ml. 2. Standard deoxyribonucleic acid (DNA) curves at different wave lengths in the 'DU' spectropotometer prepared with chemically pure DNA. Maximum spectrophotometric absorption took place at 600 ml.

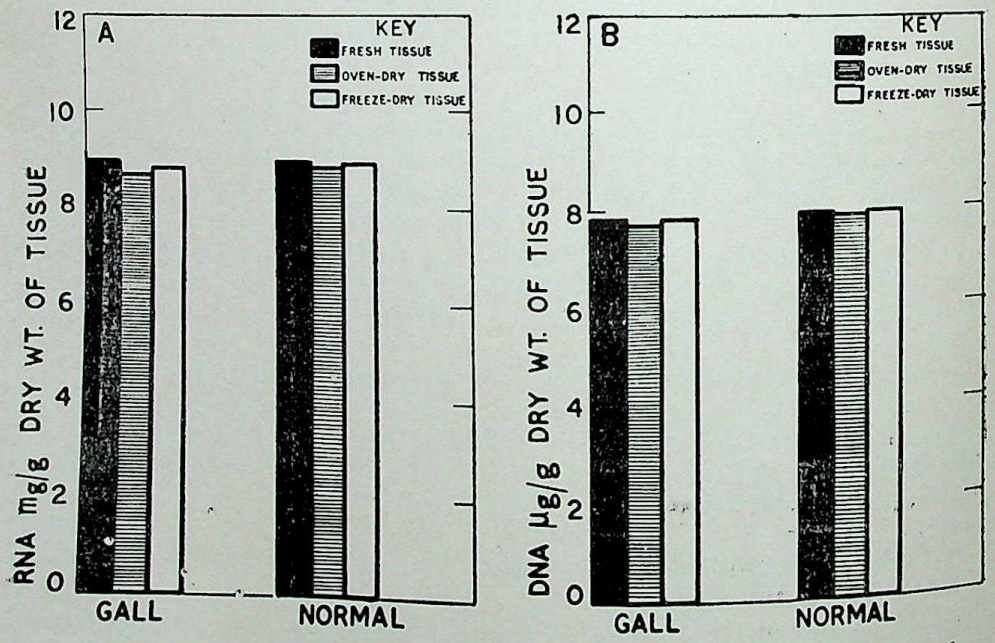


Fig. 3. Extractability of RNA and DNA under fresh, oven-dry and vacuum-freeze-dry conditions of the gall and normal callus tissues.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

in a referigerator. The solution was useful for at least six months. A stock reagent was prepared by diluting 60 ml. of the stored solution to 400 ml. with glacial acetic acid and adding 6 ml. of concentrated sulphuric acid. The stock reagent solution was kept at room temperature around 25°C. Just before use, 0.1 ml. of an acetaldehyde solution (16 mg./ml. H_2O) was added to each 20 ml. of stock reagent. Two volumes of stock reagent solution were added to one volume of appropriately diluted extract and the mixture incubated for 18 hrs. at 25°-30°C. The optical densities of the color formed during incubation were determined at 600 mμ on the 'DU' spectrophotometer.

Finally the values of RNA and DNA were calculated in terms of mg./g. dry weight of the tissue. The absorbancy of the spectrophotometer reading was compared with standard curves prepared with chemically pure RNA and DNA The maximum absorption of RNA and DNA solutions was obtained at 260 mμ and 600 mμ, respectively (Figs. 1, 2).

RESULTS : The data obtained are presented through the columns in the Fig. 3. It is clear from the results that the RNA and DNA values were similar in both the gall and normal tissue clones. Under all the three methods of processing the tissues (fresh, vacuum-freeze-dry, and oven-dry) for RNA and DNA fractionations, comparable results were obtained. Slightly lower yields of RNA and DNA under oven-dry tissue method were statistically insignificant.

Oven-dry method was preferable since it did not involve any appreciable destruction of nucleic acids during the treatment. It was relatively much easier to handle large samples of the tissues through this method. Actually even after drying the tissue samples in the oven, it was convenient to store them in the desiccators to be used appropriately for nucleic acid extraction later on.

SUMMARY

Two single-cell clones one each from grape stem and *Phylloxera* gall were used. A modification of Ogur and Rosen's chemical extraction method combined with Kupila *et al's* modification for deoxyribonucleic acid (DNA) extraction was employed. A comparative study was made about the efficacy of using fresh, vacuum-freeze-dry and oven dry (65°C) tissues for nucleic acid extraction. Ribonucleic acid (RNA) and DNA values were similar in both the tissues. Under all the three physical conditions of the gall and normal tissue the amounts of RNA and DNA extracted were comparable.

ACKNOWLEDGEMENT : The work forms part of the work done at the Department of Plant Pathology, University of Wisconsin, Madison, U. S. A. The work was supported in part by the Rockefeller Foundation, The American Cancer Society, The National Institutes of Health (U. S.) and The Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. The author expresses his sincere thanks to Professors A. J. Riker and A. C. Hildebrandt, Department of Plant Pathology, University of Wisconsin, U. S. A., for guidance and advice during the course of this work in their laboratory.

Botany Department,
University of Rajasthan. Jaipur (Rajasthan).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

ARYA, H. C. (1963) *In vitro* growth of *Phylloxera* gall and grape stem single-cell clones with inositol, naphthaleneacetic acid and nucleic acids. *Indian J. exp. Biol.* **1** : 148-153.

ARYA, H. C. (1964) Changes induced by nucleic acids in nucleic acid contents of *Phylloxera* gall and grape stem single-cell clones in tissue culture. *Indian J. exp. Biol.* **2** : 44-48.

ARYA, H. C., A. C. HILDEBRANDT AND A. J. RIKER (1962) Nucleic acids in callus tissues from grape stem and *Phylloxera* gall. *Phyton* **19** : 27-29.

BRAUN, A. C. (1954) The Physiology of plant tumors. *Ann. Rev. Plant Physiol.* **5** : 133-162.

KLEIN, R. M. (1958) Activation of metabolic systems during tumor cell formation. *Proc. Natl. Acad. Sci.* (U. S.) **44** : 350-354.

KLEIN, R. M., E. M. RASCH AND H. SWIFT (1953) Nucleic acids and tumor genesis in broad bean. *Cancer Research* **13** : 499-502.

KUPILA, SIRKKA, A. M. BRYAN AND H. STERN (1961) Extractability of DNA and its determination in tissues of higher plants. *Plant Physiol.* **36** : 212-215.

MARTIN, E. M. AND R. K. MORTON (1956) The chemical composition of microsomes and mitochondria from silver beet. *Biochem. J.* **64** : 221-235.

OGUR, M. AND H. ROSEN (1950) The nucleic acids of plant tissues. I The extraction and estimation of DNA and RNA. *Arch. Biochem.* **25** : 262-276.

RASCH, E., H. SWIFT AND R. M. KLEIN (1959) Nucleo-protein changes in plant tumor growth. *J Biophys. and Biochem. Cytol.* **6** : 11-34.

ROBSON, H. H., M. A. BUDD AND H. T. YOST Jr. (1959) Comparison of the nucleic acid, total nitrogen, and protein nitrogen levels of normal and tumor tissue under similar growth conditions. *Plant Physiol.* **34** : 435-440.

SCHMIDT, G. AND S. J. THANNHAUSER (1945) A method for the determination of DNA and phosphoproteins in animal tissues. *J. Biol. Chem.* **161** : 83-89.

SKOOG, F. (1954) Substances involved in normal growth and differentiation of plants. In abnormal and pathological plant growth. Brookhaven symposia in biology. **6** : 1-21.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

WOODSTOCK, L. W. AND F. SKOOG (1960) Relationship between growth rates and nucleic acid contents in the roots of inbred lines of corn. *Amer. J. Bot.* **47** : 713-716.

WOODSTOCK, L. W. AND F. SKOOG (1962) Distribution of growth, nucleic acids, and nucleic acid synthesis in seedling roots of *Zea mays*. *Amer. J. Bot.* **49** : 623-633.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ADDITIONAL VIRAL DISEASES OF TEMPERATE FRUITS IN SIMLA HILLS

B. B. NAGAICH[1] AND K. S. VASHISTH[2]

(Accepted for publication August 10, 1965)

Although more than 50 viruses are known to affect temperate fruits in western countries, only a few of them have been recorded in India (Bhargava and Bisht, 1957 ; Azad and Sehgal 1958 ; Bisht and Gupta, 1962 ; Nagaich and Vashisth, 1962). Some additional diseases were observed on apple, cherry, and almond trees during surveys of hilly regions of Punjab and Himachal Pradesh and are being reported in this paper. Since isolations made from these materials did not reveal the presence of any pathogenic organism and since the symptoms resembled those of viral diseases, mechanical and graft transmissions were made in a glasshouse at Simla. For mechanical transmission, the extracts from the leaves of the affected plants were inoculated on the respective fruit seedlings as well as some herbaceous plants. Wedge grafting was carried out during February-March when the fruit plants were in dormant condition. Bud grafts were made during May-June when the plants were growing fast.

Little leaf of apple (*Pyrus malus* Linn.) :

Little leaf of apple was recorded on Red Delicious variety of apple in Punjab hills and Mashobra (H. P.) areas. Although the disease could not be transmitted by sap inoculation, it was readily transmitted by wedge grafting to Red Delicious and Lord Lambourne varieties of apple. The symptoms consisted of the smalling of the leaves and internodes (Fig. 1). Leaves were mottled and deformed (Fig. 2). Healthy looking branches were also present among the diseased ones. Leaf buds on the affected branches open later than the buds on health of branches. Some of the diseased buds do not open at all and tend to die eventually. Further studies on the identity of the causal virus are in progress.

Leaf pucker of apple (*Pyrus malus* Linn.) :

Leaf pucker and chlorotic spot disease of apple was first recorded at the Regional Fruit Research Station, Mashobra (H. P.), on Golden Delicious apple. Later, the disease was also observed in several other areas on Golden Delicious, Mc Intosch, Northern Sinap and Staymen Wine Sap varieties. Besides the above mentioned varieties, the disease has been graft - transmitted to Jonathan and Royal Delicious varieties of apple. The first symptoms are light yellow spots and flecking near

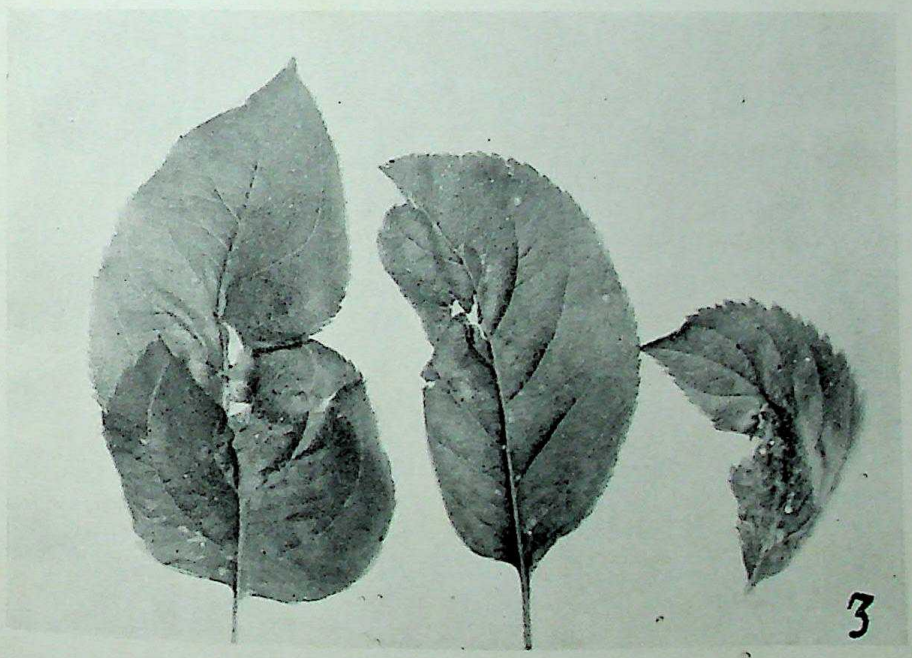
[1]Virus Pathologist, Central Potato Research Institute, Simla-1.

[2]Assistant Virus Entomologist, Plant Virus Research Sub-station, Poona.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

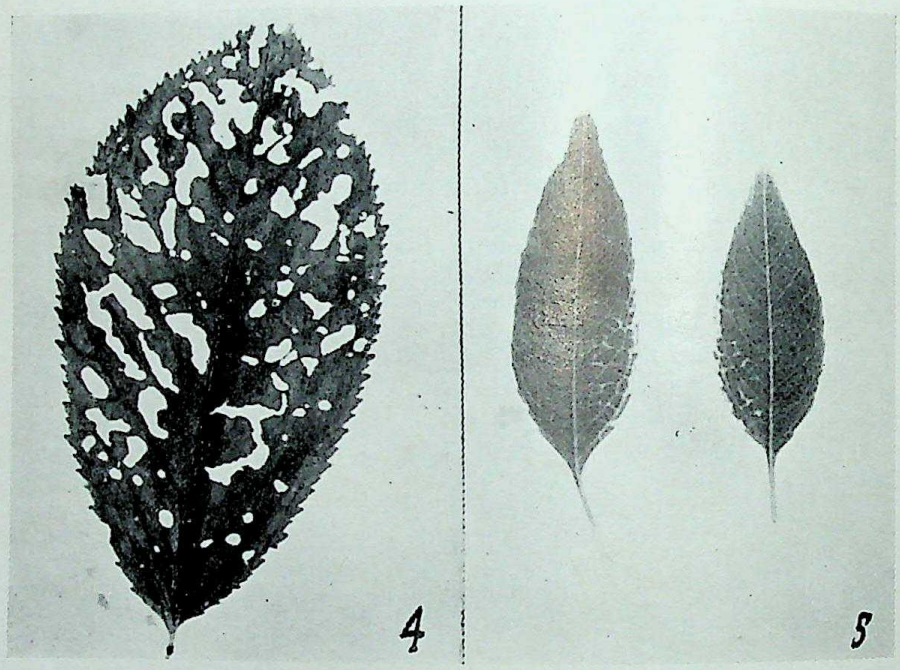
Fig. 1—2. 1. Smalling of the leaves and internodes of Red Delicious apple. 2. Mottling, deformity and reduction of lamina.



3. Puckering and deformity of apple leaves.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 4—5. 4. Tattered leaves of Babock cherry. 5. Yellowing of the veins of almond leaves.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the veins. These symptoms are prominent on the leaves formed during early part of the season. Subsequently, the spots become necrotic and the leaves are puckered and deformed (Fig. 3). The disease resembles the one reported in Canada by Welsh and Keane (1957).

Tatter leaf of sweet cherry (*Prunus avium* Linn.) :

Tatter leaf of cherry is common in the hilly regions of Himachal Pradesh and Punjab. Both cherries and peaches are affected with this disease. It has been transmitted to seedlings of peach varieties Alton and Elberta and sweet cherry variety Babcock by wedge and bud-grafts. *Prunus padus* (Bird's cherry) has also been found to be susceptible to the causal virus. The disease has been transmitted mechanically from peach to squash (*Cucurbita maxima* Duchesne.) var Butter nut. Mechanical transmission back to peach or from peach to peach or cherry has not been successful so far. The affected squash plants are stunted and the leaves show typical mosaic mottling. Initial symptom of the disease on cherry and peach is the production of chlorotic ring spots, which later become necrotic. After sometime, the necrotic tissues fall down leaving the holes in the leaves. The holes are of different shapes and sizes (Fig. 4). This disease closely resembles with the Tatter leaf of cherry reported from U.S.A. (Willison, Berkeley and Hilderbrand, 1951).

Yellow vein of almond (*Prunus amygdalus* Basteh.) :

Yellow vein of almond was recorded on Kagzi almond plants in Stone Fruit Nursery at Simla. This disease is not sap transmissible but it could be transmitted to almond by means of bud grafts. The first indication of the disease is yellow flecking at the margin of the leaves formed during early season. Later, the veins become yellow and characteristic 'Yellow net' of veins develops on a part or the entire lamina (Fig. 5). Sometimes, whole or a part of leaf blade becomes yellow. A similar disease of almond had been reported by Scaramuzzi (1956) from Italy and by Bisht and Gupta (1962) from Kumaon Hills.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his valuable suggestions and to Dr. S. P. Raychaudhuri, Professor of Plant Pathology, for kindly going through the manuscript critically. Thanks are also due to the staff of the Regional Fruit Research Station, Mashobra (H. P.), for the help provided during these investigations.

Indian Agricultural Research Institute,
Plant Pathological Sub-Station,
Flowerdale, Simla-2.

REFERENCES

AZAD, R. N. AND O. P. SEHGAL (1958) Some virus diseases of temperate fruits. *Indian Phytopath.* **11** : 159-164.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BHARGAVA, K. S. AND N. S. BISHT (1957) Three virus diseases of hill fruits in Kumaon. *Curr. Sci.* **26** : 324-325.

BISHT, N. S. AND S. C. GUPTA (1962) Two more virus diseases of fruit trees from Kumaon. *Sci. and Cult.* **28** : 178-179.

NAGAICH, B. B. AND K. S. VASHISTH, (1962) On the occurrence of virus diseases of temperate fruits in India. *Indian Phytopath.* **15** : 222-224.

*SCARAMUZZI, G. (1956) (Symptomatological polymorphism of virus complex of Almond known as mosaic in Apulia. (Part I.). *Ann. Sper. Agr. N. S.* **10** : 1707-1143.

WELSH, M. F. AND F. W. L. KEANE (1957) Leaf pucker—a virus disease of apple. *Proc. Canad. Phytopath. Soc.* **25** : 18.

WILLISON, R. S., G. H. BERKELEY AND E. M. HILDERBRAND (1951) Tatter Leaf (*in Virus Diseases and Other Disorders With Virus Like Symptoms of Stone Fruits in North America*). U. S. D. A. Handbook N. 10, Washington D. C.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE STIMULATION OF CONIDIAL GERMINATION IN COLLETOTRICHUM FALCATUM*

PRITAM SINGH

(Accepted for publication August 10, 1965)

INTRODUCTION : Studies have shown that the conidia of *Colletotrichum falcatum* Went under normal laboratory conditions (temperature 27°C and relative humidity 100 per cent) exhibit poor germination. The light type highly virulent isolates show about 12—18 per cent and the dark type weakly pathogenic ones 31-38 per cent germination (Singh, 1962). In view of such a low percentage of germination, it was desired to investigate into the possibility of improving the same. Cochrane (1958) has reviewed voluminous literature on the improvement of germination of fungal spores which can be achieved by the addition of some nutrient or non-nutrient chemicals, or by altering certain physical conditions, like temperature and humidity. The spores are subjected to such conditions before or during germination with the result that some changes are brought about in the spore contents or the spore wall and the spores are induced to germinate. In an attempt to enhance the germination of conidia of *C. falcatum*, the effect of certain physical treatments like pre-soaking, alternate wetting and drying, temperature, and afew chemicals has been studied, the results of which are reported in this paper.

MATERIAL AND METHODS : Single-spored, actively growing cultures (12-16 days' old) of seven typically light type highly virulent isolates (Nos.** 204, 301, 304, 357, 382, 390 and strain "I") and three typically dark type weakly pathogenic ones (Nos.**7, 78 and 300) were used in the present investigations.

The germination for each isolate was studied in standard conidial*** suspensions (15-20 spores per microscopic field under the low power of microscope) in moist chambers, made out of pairs of sterilized Petri plates at 27°C**** after an incubation of 18 hours****. The criterion for germination of a conidium was the emergence of a germ tube to a length exceeding its width. Observations were recorded for each experiment both on the percentage of germination and the length of the germ tube and the average data is based on about 3000 spore counts of 3 repetitions of the experiment. Figures within brackets show the range of germination percentage.

---

*The work reported herein is a part of dissertation submitted by the author for Ph D. degree in Plant Pathology at the Indian Agricultural Research Institute, New Delhi, India.

**Indian Type Culture Collection.

***The harvested conidia were freed off adhering external nutrients and mycelial fragments through washing them twice in sterile distilled water by centrifugation (2800 rpm for 5 min., each).

****Optimum temperature and incubation period (Singh, 1962).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL RESULTS : *Physical Treatments* : Considerable study has been done on the effect of certain physical factors like pre-soaking, alternate wetting and drying, and high temperature treatment, which in some way has been reported to stimulate germination. Similar studies have been taken up with *C. falcatum* conidia.

Effect of pre-soaking : Pre-soaking of conidia was done in 10 ml. of distilled water for 48 hours after which the water was decanted off and the conidia used for making standard suspension to study germination.

TABLE 1 : Effect of pre-soaking (A) and alternate wetting and drying (B) on the germination of conidia of the ten isolates of *C. falcatum*.

C. falcatum Isolate No.	Germination percentage		
	Treatment A	Treatment B	Control
244	29.3 (24.3-33.3)	24.8 (19.6-30.7)	14.8 (11.8-17.0)
357	29.0 (27.3-32.3)	26.9 (21.1-32.1)	13.1 (11.7-15.0)
"I"	44.5 (42.0-46.5)	44.9 (38.3-55.7)	22.4 (19.1-24.5)
390	36.5 (32.3-44.1)	22.4 (20.7-24.4)	16.4 (14.8-17.6)
301	26.8 (23.1-30.0)	17.5 (15.2-20.4)	13.9 (12.3-16.2)
304	51.3 (46.6-54.7)	43.0 (32.1-52.9)	24.2 (21.7-25.8)
382	18.4 (16.2-22.0)	16.2 (15.2-18.4)	7.3 (5.2-9.7)
300	51.1 (42.6-56.2)	47.6 (42.5-50.8)	38.5 (34.2-43.9)
78	45.5 (39.8-51.0)	48.3 (42.8-54.5)	35.7 (30.3-41.3)
7	45.7 (39.4-51.7)	37.3 (22.9-45.3)	37.4 (32.6-40.1)

Effect of alternate wetting and drying : It was achieved by soaking the conidia in 10 ml. of distilled water for 24 hours, followed by 24 hours' drying on a glass slab at room temperature (temperature 40°C and relative humidity 27 per cent). Such alternate wetting and drying was repeated four times and the germination of the treated conidia studied.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results obtained (Table 1) show considerable stimulation of the treated conidia.

Effect of high temperature treatment : Concentrated conidial suspensions of the isolates were prepared, separately, in distilled water and each divided into seven equal parts. Six of such portions were heated to six different temperatures in a water bath, *i. e.* 40°, 45°, 50°, 55°, 60° and 70°C., respectively, for 5 minutes, each. The supernatant was decanted off and standard suspensions of the treated conidia were prepared in fresh distilled water and their germination studied. The seventh portion of the suspension (unheated) served as a control.

TABLE 2 : Effect of preheating on the germination of the conidia of the ten isolates of *C. falcatum*

C. falcatum Isolate No.	Germination percentage				
	40°	45°	50°	55°	Control
244	48.1 (45.4-50.9)	64.1 (62.8-65.4)	27.8 (22.3-33.3)	0	13.6 (12.1-15.2)
357	47.8 (44.1-51.6)	71.5 (69.6-73.5)	29.6 (27.5-31.7)	0	12.0 (10.6-13.4)
"I"	55.6 (50.0-56.3)	74.0 (71.3-76.6)	40.1 (34.5-45.7)	7.2 (0.0-14.4)	22.8 (21.5-24.2)
390	57.2 (53.3-61.1)	72.1 (71.6-72.6)	28.8 (15.0-42.6)	14.3 (8.3-20.3)	17.7 (17.3-18.2)
301	50.9 (49.2-52.6)	63.1 (61.3-65.0)	26.2 (19.2-34.0)	5.4 (0.0-10.8)	18.8 (18.4-19.2)
304	58.0 (57.5-58.6)	79.2 (78.5-80.4)	36.6 (36.2-37.0)	13.0 (9.1-17.0)	22.6 (22.1-23.1)
382	47.5 (46.6-48.4)	67.2 (66.1-68.3)	15.1 (13.1-17.1)	0	8.0 (7.0-9.0)
78	68.9 (68.4-69.5)	81.1 (77.5-84.7)	37.8 (36.6-39.0)	14.5 (0.0-29.0)	35.7 (35.3-36.2)
300	67.4 (63.3-71.6)	87.1 (86.6-88.5)	40.0 (31.6-48.4)	16.7 (13.0-20.4)	42.2 (41.4-43.1)
7	66.2 (58.4-74.0)	84.5 (79.6-89.4)	37.9 (37.4-38.5)	0	36.1 (31.7-40.0)

The results (Table 2) show that heating of conidia to 40°, 45° and 50°C has a definite stimulatory effect, maximum germination being after heating to 45°C. At 60°C. and above, the spores appear to have been killed as they failed to germinate. Isolate Nos. 244, 357 and 382 of the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

light type and isolate No. 7 of the dark type could not withstand pre-heating even up to 55°C. However, in other isolates the germination was reduced considerably at this temperature.

In all these treatments, *i. e.* presoaking, alternate wetting and drying and heating of conidia, the effect was more pronounced on the percentage of germination, with better response from the light type isolates.

Effect of the extract of heated Spores : An attempt to study stimulation through the extract of heated spores was also made. Concentrated conidial suspensions of the seven isolates (isolate Nos. 7, 78, 244, 300, 301, 382 and strain "I") were prepared, separately, in 20 ml. of distilled water and heated to 80°C for 10 minutes in a water bath. The supernatant liquid was decanted off into another clean test tube, filtered once through whatman filter paper No. 1 and used for making the standard suspensions of fresh conidia in such a way that each isolate was germinated in its respective extract.

TABLE 3 : Effect of the extract on the germination of conidia of the seven isolates of *C. falcatum*

C. falcatum Isolate No.	Germination percentage	
	Treatment	Control
244	81.2 (77.2-84.0)	16.6 (11.1-20.4)
"I"	85.7 (84.1-89.5)	23.8 (22.1-27.5)
301	82.0 (79.6-84.7)	15.0 (13.1-17.1)
382	73.7 (69.5-78.7)	8.4 (5.3-11.0)
78	75.7 (72.6-80.6)	39.3 (36.2-42.7)
300	83.1 (77.7-87.6)	40.6 (38.2-43.0)
7	74.1 (66.1-79.6)	36.1 (34.0-37.4)

The results (Table 3) indicate that some substance or factor is present in the extract, which has diffused out of the spores on heating and has stimulated the conidial germination considerably. The effect is mostly on the percentage of germination, more pronounced in the light type isolates.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 4 : Effect of germinating the conidia of the eight isolates of *C. falcatum* in different chemicals.

C. falcatum Isolate No.	Germination percentage					
	Furfural	EDTA	Fumaric acid	Oxalic acid	Citric acid	Control
244	67.0 (60.1-74.0)	90.9 (86.1-95.7)	42.2 (40.8-43.7)	12.5 (11.7-13.4)	14 3 (13.3-15.4)	12.1 (11.0-13.1)
"I"	63.2 (55.0-71.4)	84.3 (81.3-87.3)	45.7 (44.1-47.4)	22.0 (20.2-23.8)	26.3 (25.5-27.3)	19.4 (18.4-20.4)
301	59.1 (58.6-60.7)	82.3 (79.6-85.1)	43.8 (41.1-46.6)	17.7 (16.2-19.2)	16.0 (15.9-16.2)	16.0 (15.0-17.1)
304	68.8 (64.7-73.0)	88.7 (88.0-89.4)	45.7 (44.1-47.4)	27.7 (25.4-30.0)	23.4 (21.6-25.4)	22.5 (22.1-22.9)
382	55.0 (54.4-55.7)	90.3 (88.5-92.2)	46.9 (46.5-47.4)	14.3 (14.1-14.5)	12.1 (11.6-12.6)	10.0 (9.1-11.0)
78	70.0 (68.4-71.6)	76.2 (74.0-78.5)	55.1 (54.0-56.3)	39.8 (39.6-40.0)	40.7 (40.1-41.3)	40.3 (39.0-41.7)
300	70.2 (69.5-71.0)	80.1 (79.6-81.7)	62.3 (59.3-65.4)	42.8 (41.4-44.2)	42.1 (40.0-44.2)	41.9 (40.8-43.0)
7	66.6 (60.6-72.6)	68.3 (64.1-72.6)	50.5 (49.1-52.0)	39.5 (39.0-41.0)	38.8 (38.5-39.1)	35.5 (34.0-37.0)

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 5 : Effect of water-soaking on the germination of conidia of the eight isolates of *C. falcatum* in different chemicals.

C. falcatum Isolate No.	Germination percentage						
	Furfural	EDTA	Fumaric acid	Citric acid	Oxalic acid	Control (Soaked)	Control (Unsoaked)
244	90.7 (90.0-91.4)	93.7 (93.2-94.2)	70.0 (68.1-72.0)	33.7 (28.5-39.0)	24.4 (15.6-33.3)	25.3 (23.1-27.5)	13.1 (11.8-14.4)
"I"	88.7 (87.4-90.1)	92.7 (92.1-93.4)	73.8 (71.4-76.3)	40.2 (36.3-45.1)	39.0 (31.5-46.6)	43.7 (40.8-46.6)	22.9 (22.1-23.8)
301	81.9 (80.1-83.8)	86.4 (84.6-88.2)	62.2 (60.1-64.3)	32.6 (31.1-34.1)	25.7 (21.1-30.4)	33.1 (32.1-34.2)	16.0 (15.0-17.0)
304	81.1 (75.4-86.8)	90.8 (90.3-91.6)	65.3 (63.4-67.2)	51.8 (47.3-56.4)	44.4 (33.7-53.2)	52.5 (50.2-54.8)	25.8 (25.0-26.6)
382	56.1 (50.8-61.4)	90.0 (87.2-92.8)	63.6 (59.2 68.0)	21.2 (19.1-23.4)	20.3 (16.2-24.5)	20.2 (18.4-22.0)	7.6 (7.1- 8.2)
78	73.1 (70.7-75.5)	76.4 (75.2-77.7)	72.5 (70.6-74.4)	50.5 (50.4-50.6)	54.2 (52.3-56.1)	55.3 (54.7-56.0)	36.9 (35.7-38.2)
300	79.7 (78.0-81.5)	83.5 (82.6-84.5)	76.2 (73.3-79.1)	55.1 (53.1-57.2)	54.1 (54.0-54.2)	52.2 (51.6-52.9)	42.8 (41.8-43.9)
7	74.3 (71.4-77.2)	73.7 (73.0-74.4)	67.8 (65.3-69.4)	50.3 (44.5-56.1)	54.5 (51.1-58.0)	48.1 (46.0-50.3)	37.1 (34.6-39.7)

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It now appears that as a result of heat treatment, the stimulation is not only due to some changes within the spore or the spore wall, but also due to some substance which diffused into the water on heating the conidia.

Chemical treatments : Chemicals, like ethyl diamine tetra-acetic acid (EDTA), fumaric acid, oxalic acid and citric acid, already known to be stimulatory to fungal spores, were used at a dilution of 10 ppm. Furfural, however, was tried at 1 ppm. dilution. Germination response of the eight isolates (Nos. 7, 78, 244, 300, 301, 304, 382 and strain "I") was studied in three different ways :

(a) Germination of conidia directly in the chemicals.

(b) Germination of water-soaked conidia in the chemicals.

(c) Germination of chemical-soaked conidia in distilled water.

In the experiment (b), the conidia were soaked, in distilled water for 24 hours before germinating them in the chemicals. However, in (c), the conidia were soaked in these chemicals and then germinated in distilled water. Suitable controls were run in all the three cases. In (b), two controls, one with soaked and the other with unsoaked conidia, were run in each case.

TABLE 6 : Effect of chemical-soaking on the germination of conidia of the eight isolates of *C. falcatum* in distilled water.

C. falcatum Isolate No.	Germination percentage					
	Furfural	EDTA	Fumaric acid	Oxalic acid	Citric acid	Control (Unsoaked)
244	59.7 (55.2-64.3)	79.9 (75.7-84.2)	40.7 (40.0-41.5)	0	0	12.1 (12.0-12.2)
"I"	57.0 (52.7-61.4)	68.8 (63.6-74.0)	48.6 (48.2-49.0)	0	0	20.1 (19.4-21.0)
301	63.5 (57.0-70.1)	78.9 (72.1-85.7)	43.1 (42.1-44.1)	0	0	13.7 (13 3-14.1)
304	67.2 (63.3-71.2)	84.2 (81.2-87.2)	41.1 (39.0-43.2)	0	0	22.7 (22.4-23.1)
382	51.5 (51.0-52.0)	84.2 (84.1-84.4)	44.4 (42.4-46.5)	0	0	7.8 (7.5- 8.1)
78	64.1 (62.2-66.0)	76.0 (74.5-77.5)	52.2 (50.0-54.4)	0	0	39.2 (36.7-41.7)
300	68.7 (67.3-70.1)	83.4 (80.3-86.6)	58.7 (57.4-60.0)	0	0	42.4 (40.7-44.1)
7	60.7 (60.2-61.3)	78.5 (75.5-81.5)	49.1 (46.5-51.8)	0	0	37.5 (35.1-40 0)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The data for these three experiments (a, b and c) are given in Tables 4, 5 and 6, respectively. It is observed that in the first two cases, some of the chemicals were highly stimulatory to germination, while the others did not have any appreciable effect. The effect is more pronounced in treatment (b). There was enhanced germination in treatment (b) as compared to that in the treatment (a). The response of all the eight isolates was similar. EDTA exhibited greatest stimulation, followed by furfural, fumaric acid and citric acid. No such stimulation was noticed with oxalic acid.

With regard to the effect of the chemicals on the length of the germ tube, it was observed that the results were not concordant with those obtained with the germination percentage. Stimulation, though, very little was observed in furfural and fumaric acid. However, there was great retardation in the growth of the germ tube in EDTA, citric acid and oxalic acid.

In experiment (c), there was observed good germination of conidia presoaked in some of the chemicals, while no germination occurred in other cases. Maximum germination was observed in the case of EDTA-soaked spores, though, comparatively, it was less than that observed in treatment (a), where the conidia were actually germinated in EDTA. Similar results were obtained with the spores treated with furfural and fumaric acid. There was no germination in the case of conidia soaked in oxalic or citric acid. The light type isolates as usual showed comparatively better response.

Discussion : Presoaking as well as alternate wetting and drying of the conidia induced marked stimulation in germination, the effect being more pronounced on the percentage of germination. Noble (1923), Gilbert (1929), Raeder and Bever (1931), Kauffman (1934-1935), Smart (1937), Cotter (1940), Lowther (1948), and Lilly and Barnett (1951) reported that alternate wetting and drying, as well as presoaking has a purely mechanical effect, i. e. softening of the spore wall. Lilly and Barnett further assumed that delayed germination is due to the impermeability of the spore membrane.

As observed in the present investigations, the light type highly virulent isolates are more sensitive to such factors, which effect a change in the permeability ; it indirectly indicates that the conidia of the dark type isolates are more resistant to such changes. Such an alternate wetting and drying, and presoaking of the conidia of the light type isolates in nature might explain the greater severity of the disease soon after the rains. It may be possible that rains, beside lowering the temperature and providing the necessary humidity, give a stimulatory treatment by presoaking, which increases germination and hence result in more severe infections. The latter is true in view of Turner's (1957) report that increased vigour of germination leads to greater chance of successful parasitic invasion.

Considerable stimulation in conidial germination was also observed after a few minutes' exposure of the conidia to temperatures above optimum, i. e. 40°, 45° and 50°C, particularly 45°C. Noble (1924), Smart

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(1937), and Lilly and Barnett (1951) believed that high temperature hastens the maturity, beside causing an increase in permeability of the spore wall as well as of the spore contents resulting in quicker ingress of water and hence resulting in better germination. Shear and Dodge (1927), Goddard (1935, 1939), Goddard and Smith (1938), and Evans and Curran (1943) reported that preheating to 50°C or more for a few minutes broke the dormancy and induced 100 per cent germination in 2 or 3 hours in *Neurospora tetrasperma*. As we do not have any knowledge of the existence of dormancy in *C. falcatum*, the assumption of Noble (1924), Smart (1937) and Lilly and Barnett (1951) seems plausible in the present investigations and it appears that the mechanism of stimulation in all the isolates is similar. In view of this, it can be expected that if the conidia of *C. falcatum*, are exposed to severe summer temperatures in nature, severe infection could be expected in the following season. Greater response of the light type isolates indicated chance for greater virulence and hence greater severity of the disease.

Investigations with chemical stimulants have shown that EDTA is the best stimulator, followed by furfural, fumaric acid, citric acid and oxalic acid. The results regarding germination percentage were not concordant with those of the length of the germ tube. As for instance, EDTA was highly stimulatory to the germination percentage, but inhibitory to the growth of the germ tube. The response of all the isolates is similar, it being greater in the light types. Maximum stimulation was obtained in case of conidia presoaked in distilled water, indicating that the water-softened wall is more permeable to chemicals from outside (Noble, 1924). This also supports the hypothesis of the role of the change of permeability. Susman (1954) reported that EDTA, as a chelating agent, acts first on the spore wall and then on the spore contents. It removes minerals lying on the former and those present endogenously within the latter, thereby increasing the permeability. As a result there is more ingress of water or nutrients from the outside, conversion of insoluble food to soluble form, activation of the enzyme systems resulting in increased respiration and more germination. The assumption of more ingress of water or nutrients from the outside is supported by the comparative measurements of the size of the conidia soaked in EDTA, furfural, fumaric acid and distilled water for 18 hours. Considerable increase (varying from 17-59 per cent) in the width of the spores in all the isolates was observed, maximum being in EDTA, followed by furfural, fumaric acid and distilled water. The increase in size is due to swelling of the spores because of more ingress of water or chemicals (Sussman, 1953, 1954; and Yarwood, 1936).

Duggar (1901) reported that organic acids lower the surface tension of the water and increase the germination. Price (1914), Stiles (1921), Noble (1924) and Cheo and Leach (1950), however, believed in the change of the permeability of the spore wall and the spore contents.

SUMMARY

Presoaking, alternate wetting and drying, and preheating treatments have been found to be stimulatory to germination of the conidia of all the ten isolates of *C. falcatum*, studied herein, the effect being more

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

pronounced in the light type isolates. Similar stimulation was achieved by certain chemicals, like EDTA, furfural, fumaric acid, oxalic acid and citric acid. It is assumed that the observed stimulation is due to an increase in the permeability of the spore wall and spore contents.

ACKNOWLEDGEMENTS : Gratefulness is acknowledged to Dr. B. L. Chona under whose guidance this work was carried out, to Dr. D. N. Srivastava for the helpful suggestions during the course of these investigations, and to Dr. V. V. Chenulu, for the help given to me in the preparation of the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

REFERENCES

CHEO, P. C. AND J. G. LEACH (1950) The stimulating effect of dung infusion on the germination of spores of *Ustilago striiformis*. *Phytopathology* **40** : 584-589.

COCHRANE, V. W. (1958) *Physiology of Fungi*. John Wiley and Sons, Inc., New York, pp. 524.

COTTER, R. U. (1940) Unseasonable germination of Teliospores of *Puccinia graminis tritici*. *Phytopathology* **30** : 689-691.

DUGGAR, B. M. (1901) Physiological studies with special reference to germination of certain fungus spores. *Bot. Gaz.* **31** : 38-66.

EMERSON, M. A. (1948) Chemical activation of ascospore germination in *Neurospora crassa*. *J. Bact.* **55** : 327-330.

EVANS, F. R. AND H. R. CURRAN (1943) The accelerating effect of sublethal heat on spore germination in mesophyllic aerobic bacteria. *J. Bact.* **46** : 513-523.

FRAMPTON, V. L. AND P. M. MARSH (1941) Respiration of conidia of *Sclerotinia fructicola*. *Phytopathology* **31** : 9.

GILBERT, F. A. (1929) Factors influencing the germination of Myxomycetous spores. *Amer. J. Bot.* **16** : 280-287.

————(1929) Spore germination in the Myxomycetes : A comparative study of spore germination by families. *Amer. J. Bot.* **16** : 421-432.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

GODDARD, D. R. (1935) The reversible heat activation inducing germination and increased respiration in the ascospores of *Neurospora tetrasperma*. *J. Gen. Physiol.* **19** : 45-60.

————(1939) The reversible heat activation of respiration in *Neurospora*. *Cold Spr. Harb. Symp. quant. Biol.* **7** : 362-376.

GODDARD, D. R. AND P. E. SMITH (1938) Respiratory block in the dormant spores of *Neurospora tetrasperma*. *Plant Physiol.* **13** : 241-264.

KAUFFMAN, F. H. O. (1934-1935) Studies on the germination of spores of certain Basidiomycetes. *Bot. Gaz.* **96** : 282-298.

LILLY, V. G. AND H. L. BARNETT (1951) *Physiology of Fungi*. McGraw-Hill Book Company, Inc., New York and London, pp. 464.

LOWTHER, C. V. (1948) Low temperature as a factor in the germination of dwarf bunt chlamydospores. *Phytopathology* **38** : 309-310.

MEFFORD, JR., R. B. AND L. L. CAMPBELL, JR. (1951) The activation of thermophilic spores by furfural. *J. Bact.* **62** : 130-132.

NOBLE, R. J. (1923) Studies on *Urocystis tritici*, the causal organism of Flag smut of wheat. *Phytopathology* **13** : 127-139.

————(1924) Studies on the parasitism of *Urocystis tritici*, the causal organism of Flag smut of wheat *J. Agric. Res.* **27** : 451-489.

PRICE, S. R. (1914) Some studies on the structure of the plant cell by the method of darkground illumination. *Ann. Bot.* **28** : 601-632.

RAEDER, J. M. AND W. M. BEVER (1931) Spore germination of *Puccinia glumarum* with notes on related species. *Phytopathology* **21** : 767-789.

SHEAR, C. L. AND B. O. DODGE (1927) Life histories and heterothallism of the bread-mold fungi of *Monilia sitophila* group. *J. Agric. Res.* **34** : 1019-1042.

SINGH, PRITAM (1962) *Studies on the variation in* Colletotrichum falcatum Went *and their correlation, if any, with virulence*. Thesis approved for Ph. D. degree of Indian Agricultural Research Institute, New Delhi, pp. 273.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SMART, R. F. (1937) Influence of certain external factors on spore germination in the Myxomycetes. *Am. J. Bot.* **24** : 145-159.

*STILES, W. (1921) Permeability. *New Phytol.* **20** : 45-55.

SUSSMAN, A. S. (1953) The effect of heterocyclic and other compounds upon germination of ascospores of *Neurospora tetrasperma*. *J. Gen. Microbiol.* **8** : 211-216.

————(1954) Changes in the permeability of ascospores of *Neurospora tetrasperma* during germination. *J. Gen. Physiol.* **38** : 59-77.

TURNER, E. M. (1957) The effect of some amino acids on the growth of two varieties of *Ophiobolus graminis*. *J. Gen. Microbiol.* **16** : 531-533.

YARWOOD, C. E. (1936) The tolerance of *Erysiphe polygoni* and certain other powdery mildews to low humidity. *Phytopathology* **26** : 845-859.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECT OF MALEIC HYDRAZIDE ON RUSTS OF WHEAT AND BARLEY*

L. M. JOSHI

(Accepted for publication August 10, 1965)

Many investigators (Samborski and Shaw, 1957 and Samborski *et al.*, 1960 etc.) have shown that maleic hydrazide, in small concentrations, increases the susceptibility to diseases in some cereals. The chemical is known to serve as growth inhibitor (Currier, Day and Craft, 1950; Compton, 1952; Nayler and Davis, 1957; Esau, 1957) and also as a promotor of severity of many diseases like wilt, rusts and leaf spots (Simons, 1955; Richards, 1957; Samborski and shaw, 1957 and Nair, 1958 etc.). This, however, should not be considered as a rule and in certain cases increase in resistance to the disease due to the treatment of the chemical and also a differential response of host varieties has been reported (Simons, 1955; Anonymous, 1961). From the literature it appears that practically no work has been done on the effect of maleic hydrazide on *Puccinia hordei* Otth, the leaf rust of barley. The following investigations were, therefore, taken up with the rust. Side by side wheat varieties were also treated to get comparative idea of their behaviour to *P. graminis tritici* and *P. recondita*. The primary aim of these studies was to utilise maleic hydrazide as a tool in certain greenhouse studies, such as maintenance of cultures, multiplication of inoculum etc.

MATERIAL AND METHOD : Maleic hydrazide at concentrations of .019 or .02 per cent was administered through the roots. It was given at the time of emergence and the pots so treated were not watered 12 hours before and 24 hours after treatment. The varieties treated were Little Club and Agra local wheat; Agra local barley and Erie variety of barley. These varieties are usually used for maintenance of rust cultures because of their high susceptibility to them. Part of the work was done in Minnesota where the varieties were inoculated with races 15-B and 4 of *P. g. tritici* and *P. hordei*, respectively. For the work carried out in India race H_2 of barley leaf rust and race 15.C of stem rust of wheat were used.

EXPERIMENTAL RESULTS : In St. Paul (Minnesota) seedlings of wheat variety Little Club and barley variety Erie were grown in 4″ pots and varying doses from 50 to 100 ml. per 4″ pot were administered as soil drench at the time of emergence of the seedlings. Wheat seedlings were inoculated with race 15-B of black rust and barley seedlings with race 4 of *P. hordei*. The pots were not watered 12 hours before and 24 hours after the treatment. Results are summarised below in Table 1.

*A portion of the Ph. D thesis submitted in partial fulfilment of requirements of the Post Graduate School, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Response of different doses of maleic hydrazide (.0192 per cent) on the growth of the host and sporulation of the fungus at 65-75°F

Name of the host and races of rusts	Doses		
	50 ml./pot	80 ml./pot	100 ml./pot
Barley var. Erie (Race 4 of (*P. hordei*)	G. R. :—Pr. Spo. :—Slightly more than control.	G. R. :—Gd. Spo. :—Profuse. Culture maintain -ed for 6 weeks (Control 24 days at the most)	G. R. :—Gd. Even 1st leaf reduced in size. Secondary leaves show drying after 4 weeks.
Wheat var. Little Club. (*P. g. tritici* Race 15-B.)	G. R. :—Pr. Spo :—N. D. Pus :—Like Control.	G. R. :—Pr. Pus :—Bigger than control. Spo. :—Better than control.	G. R. :—Gd. Pus :—Very big. Spo :—Better than Control. Maintained for 7 weeks. (Control 4 weeks)

Symbols :—G. R.=Growth Response Spo.=Sporulation.
Pus.=Pustule.

Growth Response :—N. R.=No Response (i. e. Growth as in untreated control)
Pr.=Poor. Secondary leaves slightly reduced in length and darker in colour.
Gd.=Good. Secondary leaves of same size as first leaves. Dark green in colour.
V. G.=Very Good. Secondary leaves practically suppressed or only 1-2″ long. Even first leaves slightly reduced.
N. D.=No differences. No. differences in sporulation of treated and untreated plants.

The best response of barley and wheat varieties was with 80 ml. and 100 ml. per 4″ pots, respectively. At these doses, the first leaf of barley developed normally without any visible adverse effects. They were, however, deep green in colour and more erect. The secondary leaves grew very slowly and reminded much shorter than those of untreated ones. In case of barley variety Erie the secondary leaves become chlorotic in 4 to 5 weeks and the primary leaves remained green for about 6 weeks. On the other hand, the primary and secondary leaves in the untreated plants, under identical conditions started withering in 3 weeks.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In Erie variety of barley flecking in the treated plants appeared a day later at temperature range of 60-65°F. (15.3-18.3°C) than in the untreated plants. The plants treated with maleic hydrazide showed much better development of rust pustules with copious ring-formation. Rust cultures were successfully maintained for about six weeks on treated plants while they could not be maintained for more than 3 weeks on the untreated ones. It was estimated, on visual observations of the collected inoculum, that plants treated with maleic hydrazide produce 3 to 5 times more inoculum than the untreated ones.

Since these results were obtained at St. Paul, Minnesota (U. S. A.) and also because of the fact that maleic hydrazide is known to have differential response, it was felt necessary to repeat them under Indian conditions and compare these results using Indian varieties. Due to the non-availability of viable seed of barley variety Erie, comparative tests under Indian conditions could not be conducted. The tests were, however, repeated on Agra local barley, Agra local wheat (a highly susceptible variety of *Triticum vulgare* L.) and Little Club wheat using 0.2 per cent maleic hydrazide. The seedlings were raised in 4″ pots containing soil-compost in the ration of 75 : 25 and treated at the time of emergence. Results are summarised in Table 2.

TABLE 2 : Response of different doses of maleic hydrazide on growth of Little Club and Agra local wheat varieties and Agra local barley.

Name of the host and races of rusts	Doses				
	80 ml.	90 ml.	100 ml.	110 ml.	120 ml.
Wheat (*P. g. tritici*-15-C) Little Club.	G.R. :—Pr. Spo.:—ND.	G.R. :—Pr. Spo.:—ND.	G.R :—Fr. Spo.:—Slightly more than control.	G.R.—V. G. Spo.:-At least 4 times more. Plants live 2 weeks more than control.	G.R.—V.G. Spo.:—Like that at 110 ml.
Agra local	—do—	—do—	—do—	—do—	—do—
Barley Agra local *P. hordei*-Race H	G.R. :—N.R. Spo.:—ND.	G. R.—Pr. Spo.:—ND.	G.R.—Pr. Spo.:—ND.	G.R.—Fr. Spo..—Slightly more than control.	G.R.—Fr. Spo.:—Slightly more than control.

Symbols—same as in Table 1.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It will be seen from the data presented in Tables 1 and 2 that the response of maleic hydrazide to both wheat varieties (i. e. Agra local and Little Club) was more or less similar but in case of barley varieties differential response was obtained. It was found that Agra local barley responded poorly in respect to the increase in susceptibility of host to rust as well as in suppression of secondary leaves. On the other hand the varieties of wheat, namely Little Club and Agra local, responsed at 110 to 120 ml. per pot. In both cases, there was suppression of secondary leaves and considerable increase in sporulation of *P. g. tritici*. The first leaves were darker in colour and attained their normal length but the secondary leaves remained only about half of their normal size. With 125 ml. per pot the effect was felt even in the first leaves and they showed considerable reduction in size but the secondary leaves were almost completely suppressed.

Since maleic hydrazide is known to have a differential response (Samborski *et al.* 1960 ; Simons, 1955), another set of barley varieties namely, Bolivia, Oderbrucker and Agra local was taken and this was treated with 110 ml./pot of 0.2 per cent maleic hydrazide. While there was suppression of secondary leaves, retartation of growth and increase in susceptibility to *P. hordei* in varieties Bolivia and Oderbrucker, Agra local variety showed comparatively poor response. However in case of Agra local variety if maleic hydrazide was given in 3 doses of 50, 50 and 10 ml. on 1st, 3rd and 5th day of emergence instead of a single dose of 110 ml. at emergence, the response was better.

CONCLUSIONS : From the data presented above it will be seen that growth response of the varieties to maleic hydrazide and its effect on increased-sporulation are very closely connected phenomena. In all the cases, wherever the growth showed visible changes, such as suppression of secondary or tertiary leaves and darker pigmentation in the leaves, the sporulation of the fungus exhibited considerable increase. On the other hand varieties which did not express any growth responses with the given doses did not show any appreciable differences in sporulation as well. Varieties like Little Club, Agra local wheat and Erie of barley showed much better response than Agra local barley and Quinn. The three above mentioned varieties, which showed better responses, are used in many laboratories for maintenance of cultures of rusts. There is apparently no differences in the responses of leaf and stem rusts of wheat and in both these rusts considerable increase in sporulation was observed if the hosts showed response to maleic hydrazide treatment. The present investigations throw useful light on the maintenance of black, brown rusts of wheat (*P. g. tritici* and *P. recondita* respectively) and leaf rust of barley (*P. hordei*) This method of maintenance of cultures, apart from giving a longer lease to the hosts and consequently to the rusts as well, removes the necessity of clipping off the uninoculated secondary leaves, so very necessary otherwise for proper maintenance of rust cultures. Added to these advantages is the manifold increase in the availability of inoculum. The amount of inoculum obtained from treated plants was 3-5 times more than the un-inoculated ones. Under Indian conditions, particularly in the plains, multiplication of inoculum for field work takes a lot of glasshouse space and quite often becomes a bottle neck in expansion

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

of the work. Usually the glasshouse work cannot be started in Delhi before middle of October and inoculum for field inoculations is needed within next 6 weeks or so. The method discussed above would help a great deal in increasing adequate quantity of the inoculum of races within required period even in the limited glasshouse accommodation.

SUMMARY

Maleic hydrazide at concentrations of .019 or .02 per cent was administered through roots of wheat and barley seedlings, at the time of emergence. The doses varied from 50 ml. to 120 ml. per 4″ pots. Seedlings of wheat varieties showed considerable increase in sporulatlon of races 15-B and 15-C of *Puccinia graminis tritici*. In case of barley, with 110 ml./pot of 0.2 per cent solution, there was suppression of secondary leaves, retartation of growth and also increase in susceptibility to *P. hordei* in varieties Bolivia and Oderbrucker while Agra local barley showed poor response. However, in case of Agra local barley, if maleic hydrazide was given in 3 doses of 50, 50 and 10 ml. on 1st, 3rd and 5th day instead of a single dose of 110 ml./pot at the emergence, the increase in susceptibility was considerably more.

ACKNOWLEDGEMENT : Part of this work was done at the University of Minnesota under the guidance of Prof. (Dr.) Helen Hart and Dr. D. M. Stewart and I wish to record my most grateful thanks to them for their advice, guidance and suggestions. I am thankful to Dr. R. Prasada for going through the manuscript. Visit to Minnesota was made possible by a Fellowship grant of Rockefeller Foundation and I wish to express my gratitude for it.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

REFERENCES

ANONYMOUS (1962) Research Reports. Pest Research Institute, London, Ont. 1957-1960 : 11-16.

COMPTON, W. (1952) The Effect of Maleic hydrazide on Growth and Cell Division in *Pisum sativum*. *Torrey Bot. Club. Bull.* **79** : 205-211.

CURRIER, H. B., B. E. DAY and A. S. CRAFT (1951) Some Effect of Maleic Hydrazide on Plants. *Bot. Gaz.* **112** : 272-80.

ESAU, K. (1957) Anatomical Effect of Barley Yellow Dwarf and Maleic Hydrazide on Certain Graminae. *Hilgardia* **27** : 15-69.

NAIK, P. N. (1958) Effect of Maleic hydrazide, thiourea and 2, 4,-dinitrophenol on resistance to Flax wilt. *Phytopathology*. **48** : 288-289.

NAYLOR, A. W. AND E. A. DAVIS (1951) Maleic hydrazide as Plant Growth Inhibitor. *Bot. Gaz.* **112** : 26.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RICHARDS, L. T. (1957) Effect of Insecticides and Herbicides applied to soil on Development of Diseases. I. The seedling Disease of Barley caused by *Helminthosporium sativum*. *Canad. Jour. Plant Sci.* (Formerly *Canad. Jour. Jour. Agric. Sci.*) **37** : 196-204.

SAMBORSKI, D. J. AND M. SHAW (1957) Physiology of Host-Parasite Relations. IV. The Effect of Maleic hydrazide and indolacetic acid on the rust resistance of Khapli and Little Club wheats. *Can. J. Bot.* **35** : 449-455.

SAMBORSKI, D. J., C. PERSON AND F. R. FORSYTH (1960) Differential Effect of Maleic hydrazide on the Growth of Leaf and stem rusts of wheat. *Can. J. Bot.* **38** : 1-7.

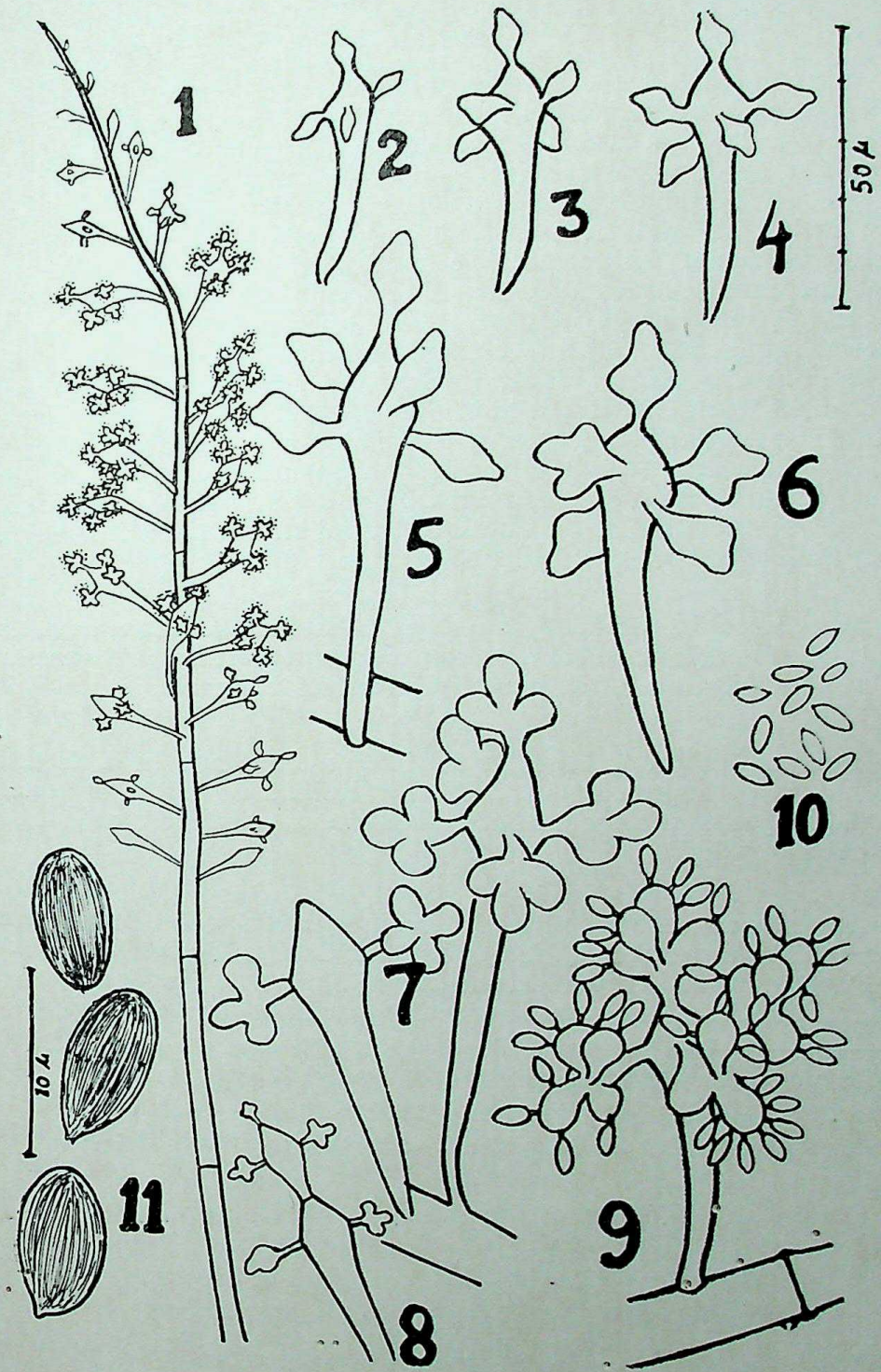
SIMONS, M. D. (1955) The use of Pathological Technique to distinguish genetically different sources of resistance to brown rust of oats. *Phytopathology* **45** : 410-413.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

On the occurrence of Botryosporium palmicolum Speg. in India.—
P. N. MATHUR AND G. SINGH, Department of Botany, Government



CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

College, Ajmer.—During a collection trip to Budha Pushkar (Rajasthan) in September 1961 a fungus was found growing in the form of white radiating hairs on the drying branches of an isolated plant of *Capsicum annum* L. On study it was found to be *Botryosporium palmicolum* Speg.[1] *B. longibrachiatum* (Oudem.) Maire.[2] is the only species of this genus which is known to occur in India[3] so far.

The conidiophores of this fungus radiate out from the surface of the host branches as white hairs of a 'bottle brush'. They are long, slender, upto 1.5 cms. in length, gradually tapering and producing lateral branches acropetally from their upper halves. Older conidiophores may also produce lateral branches in a basipetal succession from below the oldest acropetally produced branch (Fig. 1). The lateral branches (primary branches) are upto 70 μ or more long and 10-15 μ wide in the widest subterminal region, club-shaped to spearhead-shaped in appearance and each produces one terminal and four subterminal secondary branches (Figs. 2-9). The secondary branches are like the primary branches but are relatively short, 7-12.5 (or more) μ long and 5-10 μ broad in the subterminal region. Each secondary branch in turn produces one terminal and four subterminal knob-like tertiary branches, 5-10 $\times$ 6-9 μ, which then develop short sterigmata bearing the small, hyaline, oval to obovate or even elliptical and thin-walled conidia (Fig. 9). The conidia exhibit fine longitudinal striations on their surfaces and measure 7-10.5 $\times$ 3-4.5 μ (Fig. 10-11).

Sometimes the secondary branches behave in the same manner as the primary branches and elongate to produce the tertiary and quaternary branches (Fig. 8) ; the latter may then produce the spores.

For want of proper facilities this fungus could not be isolated and tested for its pathogenicity. It cannot, therefore, be said with certainty that the fungus is parasitic on *Capsicum annuum*—the host on which it was found growing.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. V. Ratnam, Associate Professor of Botany, Government College, Ajmer for his valuable suggestions and encouragement and to Shri R. S. Kapoor, Principal, Government College, Ajmer for providing facilities for work.

---

[1]Saccardo, P. A. Sylloge Fungorum **16** : 1026 (1902).

[2]Saccardo, P. A. Sylloge Fungorum **18** : 510 (1906).

[3]Subramanian, C. V. and K. Ramakrishnan, *J. Madras Univ.* B. **26** : 327-421 (1956).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A Mosaic disease of runner bean.—K. S. VASHISTH AND B. B. NAGAICH*, Indian Agricultural Research Institute, Plant Pathological Sub-Station, Flowerdale, Simla-2.—During 1962 and 1963 runner bean, *Phaseolus coccineus* L. (*P. multiflorus* Lam.) var. Kunda Fuber grown for seed multiplication in field at the plant pathological sub-station, Simla showed mosaic symptoms which were very prominent on primary leaves, but only mosaic mottle and slight deformation developed on secondary leaves. The diseased plants were stunted in comparison to healthy plants and masking of symptoms was common during winter season.

The disease was transmitted by sap inoculation to *Phaseolus vulgaris* L. var. French Yellow, Black Negro, and *Sesbania aculeata* Poir. after several attempts. All these hosts developed mild mosaic only. Ten varieties of *Phaseolus vulgaris* L. and *Lathyrus sativus* Linn. were found to be symptomless carriers.

The virus tolerated heating for ten minutes at 63°C but not at 65°C, and it remained infectious at a dilution of 1 in 6000 but lost its infectivity at a dilution of 1 in 8000. The virus *in vitro* was viable for 66 days when frozen in frigidaire but was rendered innocuous between 20 to 22 days when stored at room temperature (15—21°C).

The virus was found to be seed-borne in 42 per cent of the seeds of infected vines. *Aphis craccivora* (Koch.) and *Myzus persicae* (Sulz.) failed to transmit the disease.

The natural infection of the runner bean has been reported in the ease of two legume viruses, i. e. common bean mosaic virus from New Zealand (Chamberlain)[2] and bean yellow mosaic virus from Australia (Harvey)[3]. The common bean mosaic virus from New Zealand was also reported to be seed-borne in runner beans to the extent of 20 to 40 per cent (Chamberlain)[1]. However, the virus under report differs very much due to its limited host range and physical properties from the two above mentioned legume viruses and as such cannot be placed in the group of any of these legume viruses. The virus can be called as runner bean mosaic virus till identity and affinities of the virus are determined by further studies.

ACKNOWLEDGEMENTS : Our grateful thanks are due to Dr. B. L. Chona, Head of the Mycology Division for encouragement during the course of investigation and to Dr. S. P. Capoor, Virus Pathologist for making helpful suggestions.

Present address :

*Central Potato Research Institute, Simla.

[1]Chamberlain, E. E. *N. Z. J. Sci. Tech.* A-20 (6) : 381-8. (1939).

[2]Chamberlain, E. E. *N. Z. Deptt. Sci. Ind. Res. Bull.* 108. (1954).

[3]Harvey, H. L. *J. Dep. Agric., W. Aust.*, Ser. 3 (5, 3) : 329-336 (1956).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Helminthosporium rostratum on maize in India—T. P. BHOWMIK AND R. PRASADA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—12. A new leaf spot disease of maize was observed in the *Kharif* season of 1964 in Uttar Pradesh. In some areas the disease was found to be fairly common.

The disease appears first as small, pale yellowish, more or less elongated spots which gradually extend as longitudinal bands or stripes along the leaf area between two lateral veins. In severe cases, lesions coalesce extending across the veins to involve larger are a of the leaf blade. These lesions, which are straw coloured with light brown margins, measure 2-40×2-3 mm. in size (Fig. 1).

Isolations from these lesions yielded a *Helminthosporium* sp. which was later identified as *Helminthosporium rostratum* Drechs. This pathogen was first described by Drechsler[3] in 1923 from a leaf spot of *Eragrostis major* Host. In 1927 and again in 1928, Bunting[1,2] reported a similar fungus on maize from the Gold Coast in Africa. Later Young, Lefebvre and Johnson[4] found it inciting leaf spot of maize and some other graminicolous hosts but in India this is the first record of *H. rostratum* Drechs. attacking maize.

Pathogenicity test was done on young Ganga-1 hybrid maize seedlings and these got readily infected when water suspension of conidia made from monoconidial culture of the pathogen was sprayed on them in a water saturated atmosphere 25°—28°C.

To study the morphological character of the pathogen, infected leaf pieces were incubated in a moist chamber in an asceptic condition at 25°C. for 5—7 days and this resulted in abundant sporulation.

Conidiophores arise singly or in small groups of 2 to 3 either through the stomata or between epidermal cells. They are dark olivaceous, sometimes branched, with their swollen bases often united, measure 22-244×4—8 microns (mean=85.37×5.50 microns), 2—14 septate and bear conidia in succession near the apex.

Conidia are dark olivaceous, straight or sometimes slightly curved, widest near or somewhat below the middle, tapering moderately towards both ends, the apex is blunt and rounded, the basal end has a rounded contour with prominently protruding hilum and germinate by two polar cells. The spores are oblanceolate but often some are strongly rostrate at the tip, generally the basal septum and sometimes also the distal septum are darker and thicker than others, peripheral wall thick. The spores measure 17-139×9-20 microns (means=62.39×12.47 microns) and 2-12 septate (mean=6.27).

ACKNOWLEDGEMENTS : The authors are grateful to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for his keen interest and help.

[1]Bunting R. H., Dept. Agr. Goldcoast Year Book 1926, Bull. No. 7 : 25-27 (1927).
[2]———————., Dept. Agr. Goldcoast, Bull. No. 10 : 19-20. (1928).
[3]Drechsler, C., Jour. Agr. Res (U. S.) 24 : 722-724. (1923).
[4]Young, G. Y., Lefebvre, C. L., and Johnson, A. G., Phytopathology 47 : 180-183. (1947).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

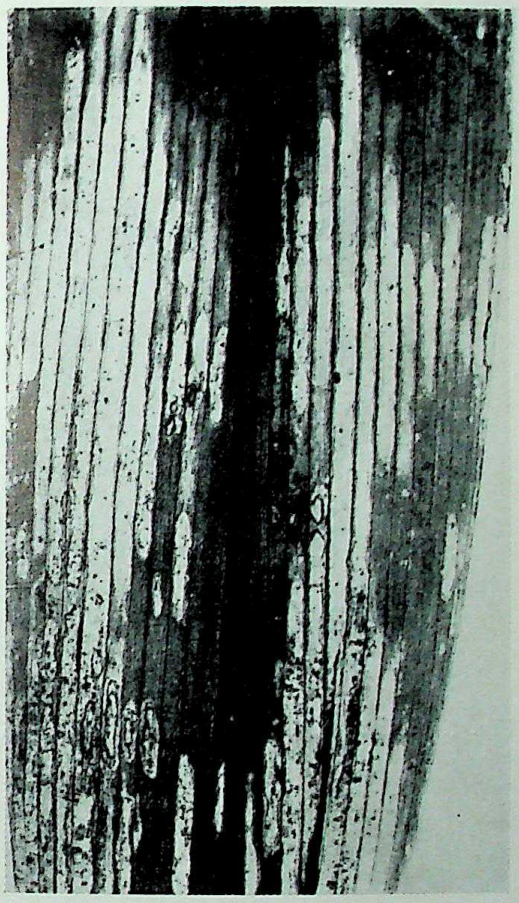


Fig. 1. Longitudinal straw coloured stripes on maize leaves due to *H. rostratum*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

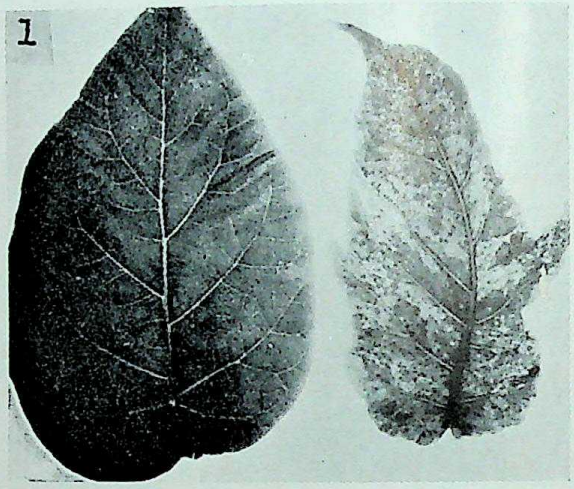


Fig. 1. Infected leaf of tree tomato showing distortion and mottling. Healthy leaf on left.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A mosaic disease of tree tomato (Cyphomandra betacea Sendt.).—R. D. JOSHI AND K. S. BHARGAVA, Department of Botany Gorakhpur University, Gorakhpur.—Recently a few plants of tree tomato were noticed showing symptoms which were quite distinct from the ones described earlier[1]. The affected plants show mosaic mottling of leaves, which are narrowed, smaller than normal and have uneven margin (Fig. 1). Such plants are slightly stunted and show sparse appearance due to reduction in amount of foliage. It appeared that a different virus was involved in this disease.

Several plants were inoculated by rubbing the leaves with the sap from diseased plants. The virus produced mosaic mottling and general stunting in *Cucumis sativus* L., *C. melo* L., *Cucurbita pepo* L., *Nicotiana tabacum* L., *N. glutinosa* L., *N. rustica* L., *Capsicum annuum* L., *Petunia hybrida* Vilm., *Datura stramonium* L., *Physalis peruviana* L., *Iberis amara* L., *Vigna sinensis* Endl., *Zinnia elegans* Jacq. and *Spinacia oleracea* L.

The physical properties of the virus were tested by the usual methods on *Nicotiana tabacum* var. White Burley test plants. The virus had the following properties "in vitro" :

Resistance to heat : between 60-65°C for 10 minutes.

Tolerance to dilution : between 1 in 10^3 - 1 in 10^4

Longevity in vitro : between 3-4 days at 20-22°C.

The virus is easily transmitted by *Myzus persicae* Sulz. and *Aphis gossypii* Glov., and is of non-persistent type. The infective sap showed positive reaction with the antiserum against cucumber mosaic virus and negative reactions with the antisera against potato virus Y, potato virus X and tobacco mosaic virus.

The symptoms produced on the differential hosts and physical properties of the virus under investigation are in general agreement with those of cucumber mosaic virus described by Smith[2]. This is confirmed by the serological test.

Cucumber mosaic virus has been noticed by the authors causing diseases in a number of other economic plants. Tree tomato is a perennial plant and is noticed to harbour different aphids, viz. *Myzus persicae*, *Aphis gossypii* along with others. Since the behaviour and spread of cucumber mosaic virus and potato virus Y in relation to these aphids is similar, they might be acting as vectors for the spread of cucumber mosaic virus also and that this plant acts as potential source of infection to other crops.

---

[1]Bhargava, K. S. and Joshi, R. D. *Amer. Potato Jour.* **36** : 288-291 (1959).

[2]Smith, K. M. A text book of Plant Virus Diseases J. and A. Churchill Ltd. London (1957).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A newphyllosticta species on sorghum in India.—D. SURYANARAYANA AND MAHENDRA PAL*, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—A leaf spot of sorghum caused by *Phyllosticta sorghiphila* Saccas was found in Broach (Gujrat State) in the last week of January 1963. This is the first record of its occurrence in India. Infection was, however, not found to be heavy.

The fungus was first described by Saccas[1] in French Equatorial Africa on sorghum and according to him it is responsible for considerable damage to leaves during wet seasons.

Spots produced by the fungus are small elongated grey to dark grey in colour measuring 1—1.5×2—10 mm. and they develop more or less parallel to the veins. Minute black pycnidia immersed to slightly erumpent are formed in the centre of the spots. These are ostiolate, globose to sub-globose measuring 53.12—205.00μ in diameter (Fig. 3). The pycnospores are borne in gelatinous matrix on small

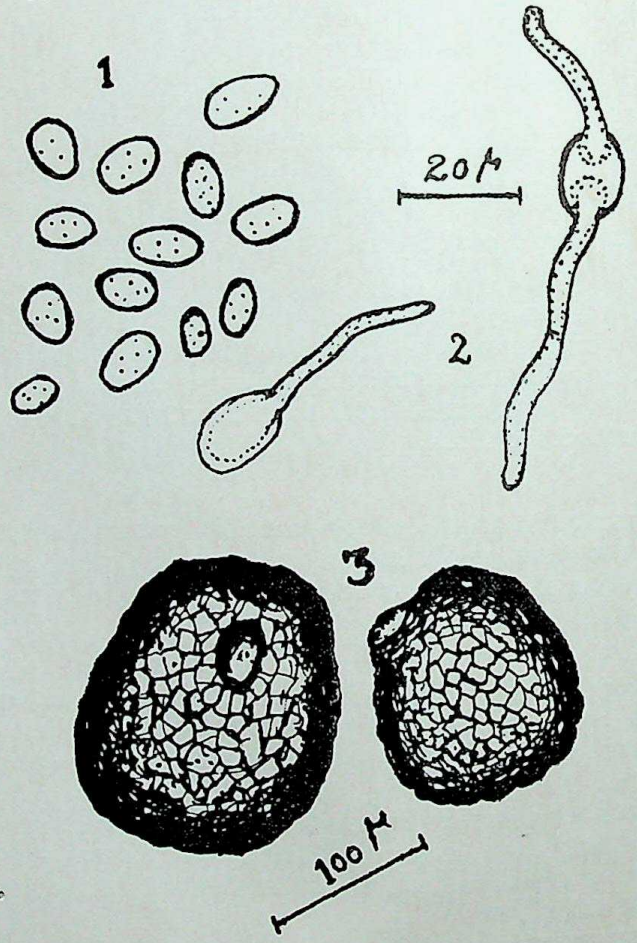


Plate 1. *Phyllosticta sorghiphila* Saccas.

1. Pycnospores. 2. Germinating pycnospores. 3. Pycnidia.

*The note is based on a part of the M. Sc. thesis submitted by the junior author to P. G. School, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sterigmata and are liberated in long cirrhi when pycnidia are put in water. The pycnospores are hyaline, unicellular, ellipsoid to oviod with rounded ends measuring $2.279-3.797 \times 6.835-9.114$ (mean $3.00 \times 7.557)\mu$ (Fig. 1). These measurements are more or less in accordance with those described by Saccas except that pycnidia are slightly larger. In *P. sorghina* Sacc. pycnidia and pyonospores are smaller and these measure $45-150\mu$ diam. and $1.7-3.4 \times 3.5-7\mu$ respectively.

Pycnospores of *P. sorghiphila*. germinate in tap water after about 8 hrs. and usually produce two germ tubes (Fig. 2). They swell slightly before germination.

ACKNOWLEDGEMENTS : The authors are indebted to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for encouragement and for giving necessary facilities. The junior author also wishes to express his gratitute to the Rockefeller Foundation for giving him the assistantship and other help during the course of this work.

[1]Saccas, A. M. *Agron. Trop. Nogent.* 9 : 263-30 (1954).

Morus Indica—An additional host of fig mosaic.—K. S. VASHISTH, AND B. B. NAGAICH*, Indian Agricultural Research Institute, Plant Pathological Sub-Station, Flowerdale, Simla-2.—Fig mosaic is of common occurrence in Simla Hills and is reported to infect three species of *Ficus*[1]. Further studies on the mode of transmission showed that the fig mite *Aceria ficus* Cotte, acts as a vector of fig mosaic virus in nature.[2] During the course of further investigations, the virus was found to be transmitted to mulberry, *Morus indica* L., by bud-grafting and not by sap-inoculation. Healthy mulberry plants, raised in glasshouse, were grafted with buds obtained from diseased fig plants in June-July. The symptoms appeared next year in March-April when new flushes came out after the winter season. The first symptoms of infection on the young leaves of *Morus indica* L. were faint chlorotic spots gradually changing to mosaic mottling of the leaves. These symptoms were apparent when the leaves were held against light. It is concluded from the above studies that *Morus indica* L. is an additional host plant of the fig mosaic virus. It has not been reported elsewhere[3].

ACKNOWLEDGEMENT : The authors are grateful to Dr. S. P. Capoor for going through the manuscript.

[1]Nagaich, B. B. and K. S. Vashisth *Curr. Sci.* **31** : 166-167 (1962).
[2]Vashisth, K. S. and B. B. Nagaich (unpublished).
[3]Burnett, H. C. *Plant Dis. Repr.* **46** : 693 (1962).
*Present address : Central Potato Research Institute, Simla-1.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Tobacco broken ring spot virus from India.—K. S. M. SASTRY*, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Smith and Markham[1] (1944) observed a virus disease producing characteristic isolated rings or ring and line patterns on the developing leaves of tobacco plants in the glass house nursery. As these rings possessed an incomplete wall *i. e.* non-necrotic areas alternating with the necrotic patches the authors named the virus as tobacco broken ring spot virus. Similar disease was consistently observed in tobacco (*Nicotiana tabacum L.* var white Burly) in glass house nurseries during the year 1962-63 and the results of investigation undertaken to establish the identity of the causal virus are presented in this communication.

Host range : Out of 37 species of plants belonging to II natural families and 22 genera, five showed systemic symptoms, only one localised reaction and none of them were found to be the symptomless carriers.

Nicotiana tabacum L. var, White Burly, and var. Harrison's Special: local lesions appeared just after 3-4 days after inoculation in the form of small single rings on the inoculated leaves. Later single rings or ring and line patterns developed on the newly formed leaves. These rings increased in number quickly so that the leaves completely become covered with a characteristic pattern. The rings have incomplete walls, non-necrotic areas alternating with the necrotic patches (Fig. 1). Infected plants exhibited general stunted growth with very little general necrosis at later stages.

Nicotiana glutnosa L. Very few necrotic spots developed in initial stages and enlarged later resulting into necrotic patches with very mild mosaic symptoms. Finally the symptoms disappeared and the plants looked apparently healthy.

Phaseolus vulgaris L. var, Canadian Wonder and var. Stringless. First the plants developed minute chlorotic areas on the inoculated leaves and few days later they became necrotic, which gradually coalesced and formed a bigger necrotic patch. A sort of mild mosaic mottling characterised with blisters of green tissue standing out between the necrotic veins giving the leaves a puckered appearance was very common in inoculated bean plants.

Cucurbita pepo DC. The virus produces innumerable chlorotic lesions on the inoculated leaves as also on cucumber (*cucumis sativa* L). These lesions are white and circular in shape and appeared in about 8-10 days time after inoculation, however, diffuse mosaic mottle which was observed in *C. sativa* was absent in *C. pepo*.

Physical Properties :

The virus was found to withstand ten minutes exposure to 55°C but not 60°C, at which temperature it was rendered innocuous. The undiluted leaf extract from the diseased plants remained infective in

*Present address : Mycology and Plant Pathology Section, Regional Research Laboratory, Canal Road, Jammu-Tawi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

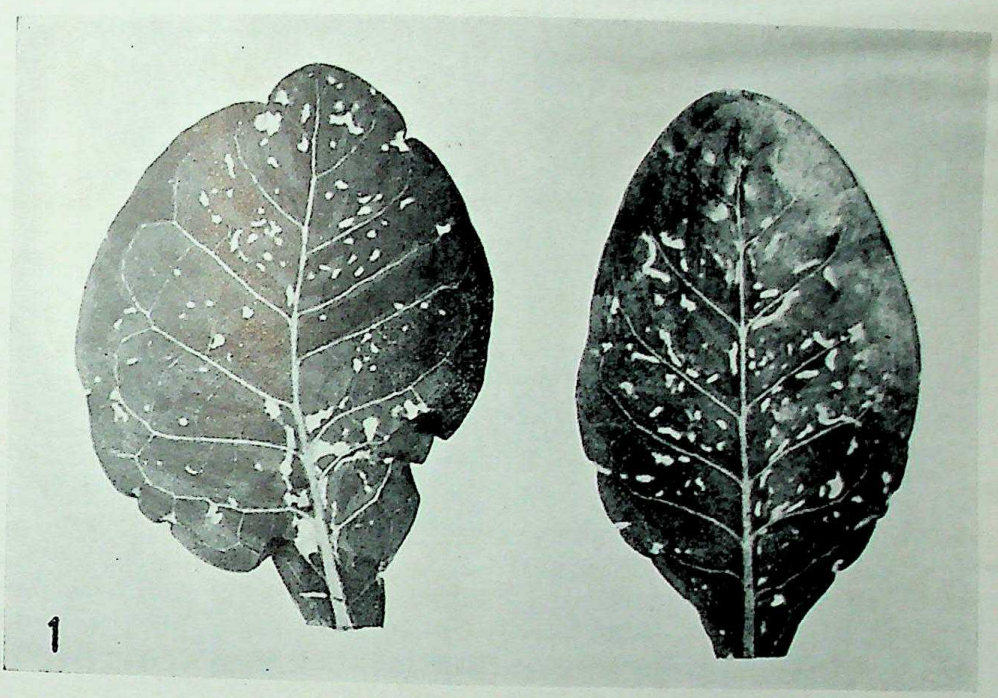
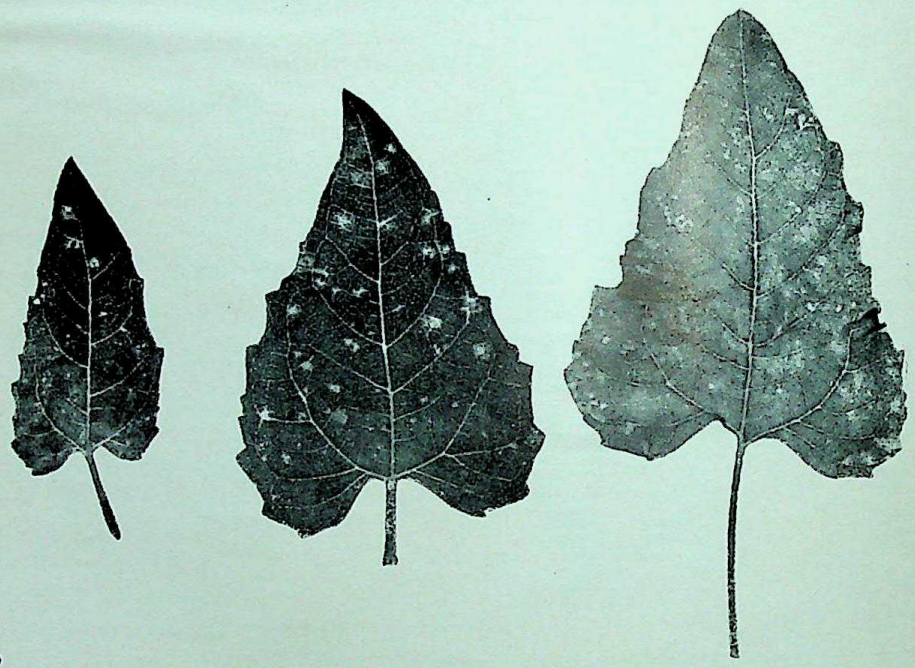


Fig. 1. Tobacco (*N. tabacum* L. var. white Burly) leaves showing broken ring symptoms.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



1

Fig. 1. Various patterns of mosaic mottle in the leaves of sunflower.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

dilutions upto 1 : 100 but not at 1 : 1000. It was found that infective sap stored at room temperature (35-37°C) retained infectivity upto the tenth day and not beyond.

Insect transmission : Few virus free insects namely *Myzus persicae* sulz., *Aphis gossypii* Glov., A. *evonymi* Fabr., *A. craccivora* Koch., *Lipaphis erysimi* kolt., *Bemisia tabaci* Gen. and a number of other unidentified insects, including different species of leaf hoppers, collected from the field failed to transmit the disease.

ACKNOWLEDGEMENTS : The author is very much indebted to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, for the facilities provided during these studies. The author is also indebted to the Council of Scientific and Industrial Research (Scientists' Pool) for the financial help.

[1]Smith, K. M. and R. Markham, *Phytopathology* 34 : 324-29 (1944).

Observations on a mosaic disease of sunflower—A. N. BATTU AND H. C. PHATAK, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Some plants of sunflower (*Helianthus annuus* L.) on the premises of the Indian Agricultural Research Institute were showing mosaic symptoms in the month of April, this year. Mosaic patterns were accompanied by ring-spots or chlorotic spots (Fig. 1) which had a tendency to coalesce. The symptoms were typical of virus infection. To study if the disease could be transmitted by means of mechanical inoculation, sap was extracted from the diseased leaves and inoculated on healthy seedlings of sunflower. Carborundum was used as an abrasive. At a temperature range of 29° to 41°C typical virus symptoms appeared in 10 days showing that the disease was caused by a virus. The virus, however, could not be transmitted to *Nicotiana tabacum* L., *N. glutinosa* L. and *Capsicum annuum* L. Although Uppal[1] has earlier recorded a mosaic disease of sunflower from India the viral nature of the disease has only now been proved experimentally.

ACKNOWLEDGEMENT : Thanks are due to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for supplying on the material and helpful suggestions.

[1]Uppal, B. N.—Inter. Bull. Plant Prot. 7 : 103-104 (1933).

Further observations and studies on pigeon pea sterility.—M. L. SETH, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—The virus causing sterility of pigeon pea (*Cajanus cajan* (Linn.) Millsp.) was transmitted by an eriophyid mite[1] which has been identified as *Aceria cajani* Channa Basavanna[2]. Some observations on the disease and further studies are being reported in this note.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In the 1956-1957 crop season survey was undertaken for the incidence of the disease at the Indian Agricultural Research Institute. It was observed that the disease incidence in the plots of mycology division was as high as 29.61 per cent as against 2.84 per cent in the plots of botany division. The incidence of the vector on examination of the leaves from healthy and diseased pigeon pea plants under the binocular microscope revealed that the population of the eriophyid mite was more on diseased plants as compared to healthy ones and their number is considerably reduced during the severe winter and summar months. Also a large number of cultivated plants and weeds grown in and around the fields at the Indian Agricultural Research Institute were examined under the binocular microscope during different seasons to find a suitable host of the vector. None of these however showed the presence of mite, *A. cajani*. Efforts were also made to colonize the mite on pigeon pea and several other crop plants under controlled conditions but without success.

Attempts were made to transmit the pigeon pea sterility virus to several crop plants viz., brinjal, cowpea, urid, mung, soyabean, *Dolichos* sp. spinach, beet, pea, groundnut and fig with *A. cajani* by picking up the mites directly from diseased pigeon pea plants by means of a single hair brush and transferring them to healthy test plants. But none of the plants showed any disease symptoms after several weeks of observations. Back inoculations from these plants could not be conducted as it was very difficult to recover *A. cajani* which is of microscopic size from the test plants after the transfer.

Cuscuta reflexa Roxb., a very common dodder plant in Delhi was successfully parasitised on pigeon pea in the glass house. Twenty transmission tests were conducted by connecting the healthy and diseased pigeon pea plants through the dodder in an attempt to transmit the disease. However, the disease could not be transmitted to any of the healthy tests plants.

Tests on reaction of pigeon pea varieties and selections to the virus were conducted by wedge grafting as well as by the agency of the mite vector. All the varieties and selections viz., NP5, NP16, NP24, NP41, NP69, UP105, S68-3A-2, S68-3A-3, S68-3A-4, S68-6B-2, S68-6B-4, S79-9-2, S79-9-3, S79-9-4, S79-9B-1, S79-9B-4, NP (WR) 15 and NP (WR) 40, which were tested in the glass house were found to be susceptible to the virus.

As *Cajanus* is a mono-typic genus comprising only *C. cajan* no other material for interspecific breeding for disease resistance or for other desirable characters is normally available. However, *Atylosia* spp. are being used in breeding programme of pigeon pea. A species of *Atylosia* I. W. 1451 available with Plant Introduction Division was therefore tested for resistance to the sterility virus. The wedge grafting was not successful due to the brittleness of *Atylosia* stem, and the transmission tests were then done with *A. cajani* but no symptoms of the disease appeared even after repeated tests. Back inoculations however from these plants to pigeon pea could not be attempted due

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

to reasons stated earlier. Thus *Atylosia* I. W. 1451 seems to be promising material for incorporating resistance to the virus.

From the foregoing observations and studies it is evident that probably neither the virus nor the vector has any other host than *C. cajan*. Both the virus and the vector seem to depend for their survival in nature on *C. cajan*. Pigeon pea is generally sown as an annual crop, but in some places it is also used as perennial crop specially for growing along hedges. Hence pigeon pea remains in the field all the year round and acts as a perpetual source of infection to the annual crops. The high incidence of disease in Mycology Division as reported above could also be attributed to the nearness of the source of infection as these plots were surrounded on one side by a perennial infected plot of pigeon pea infested with mite vector, whereas there was no other apparent source of infection in plots in Botany Division. This indicates that it may be possible to control the disease if all the old and self sown plants of pigeon pea in the vicinity of pigeon pea fields are removed well in advance of the sowing season.

ACKNOWLEDGEMENTS : The author is thankful to Dr. S. P. Raychaudhuri, Professor of Plant Pathology, I. A. R. I., New Delhi for going through the manuscript and for helpful suggestions and Dr. B. L. Chona, Head of the Division of Mycology, I. A. R. I., New Delhi for providing necessary facilities for work.

[1]Seth, M. L. *Indian Phytopath.* **15** : 225-227 (1962).

[2]Channa, B. G. P., Ph. D. thesis, Division of Entomology, Indian Agricultural Research Institute, New Delhi (1963).

A note on aphid transmission of mosaic disease of mulberry.—

S. N. CHATTERJEE* AND S. P. RAYCHAUDHURI, Indian Agricultural Research Institute, New Delhi.—In addition to the yellow net vein disease (Raychaudhuri *et al.*[2]), a mosaic disease of mulberry (*Morus indica* L.), affecting the lamina and occasionally causing slight curling and puckering of leaves has already been reported to occur at Kalimpong (Raychaudhuri *et al.*[3]). The corn aphid *Rhopalosiphum maidis* (Fitch) and *Myzus persicae* Sulz., which are very common in this region, have also been reported to be vectors (Raychaudhuri, *et al.*[3] and Chatterjee and Raychaudhuri[1]).

In the present paper the authors present data regarding transmission tests carried out with these two aphid vectors.

For transmission tests *R. maidis* and *M. persicae* were taken from colonies of healthy aphids raised on maize and tobacco plants, respectively. Apterous aphids were used in all the tests carried out in both cases and the findings of the experiments are summarised in Table 1.

*Present Address : Plant Virus Research Laboratory, Indian Agricultural Research Institute, Agricultural College Estate, Poona-5.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Transmission of mulberry mosaic by *R. maidis* (Fitch) and *M. persicae* Sulz.

Aphid species	Preacquisition fasting period in hours	Acquisition feeding period	Infection feeding period in hours	No. of aphids per plant	No. of Plants infected/ inoculated
R. maidis	0	30 mts.	24	10	2/2
	4	30 mts.	24	10	4/10
	6	24 hrs.	24	40	17/84
M. persicae	0	2-24 hrs	24	13-20	0/12
	1-2	30 mts	24	10	17/44
	6	1 hr.	24	20	13/50

From the tests carried out it was observed that *R. maidis* without any preacquisition fasting and given an acquisition feeding period of 30 minutes transmitted the disease in both the plants under test, whereas 10 aphids with a pre-acquisition fasting period of 4 hours and an acquisition feeding period of 30 minutes infected 4 out of 10 plants. Also, 40 aphids with a pre-feeding fasting period of 6 hours and an acquisition feeding of 24 hours could infect 17 out of 84 plants tested.

In transmission tests using *M. persicae* it was observed that 10 aphids given a pre-acquisition fasting period of 1-2 hrs. and an acquisition feeding period of 30 minutes transmitted the disease. It was also noticed that 20 aphids fasted for 6 hours and thereafter given an acquisition feeding period of one hour were cabable of transmitting the disease when released on healthy plants for 24 hours. In the case of 4 transmission tests the aphids were given acquisition feeding period 2 - 24 hours without any preacquisition fasting and released in groups of 13-20 aphids per plant. No transmission took place thereby indicating that a pre-acquisition fasting period is necessary before acquisition feeding period for transmission to be effective.

A comparison of the ability of transmitting the virus by the two species of aphids indicates that while *R. maidis* is capable of transmitting the virus without preacquisition fasting, *M. persicae* requires preacquisition fasting and thus indicates that it is rather a weak vector out of the two vectors studied. Since maize is extensively grown in this region and the corn aphid is of very common occurrence on this crop a careful watch should be maintained and suitable control measures adopted lest the disease is transmitted to healthy mulberry plants in the plantation.

ACKNOWLEDGEMENT : Thanks are due to Dr. B. L. Chona, formerly Head of the Division of Mycology, Indian Agricultural Research Institute, for his interest in the work.

[1]Chatterjee, S. N. and S. P. Raychaudhuri, *Indian Phytopath.* **16** : 243-44 (1963).

[2]Raychaudhuri. S. P., S N. Chatterjee and H. K. Dhar, *Indian Phytopath.* **14** : 94-95 (1961).

[3]Raychaudhuri, S. P., S. N. Chatterjee and H. K. Dhar, *Indian Phytopath* **15** : 187-88 (1962).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ABSTRACTS OF PAPERS PRESENTED AT THE INDIAN PHYTOPATHOLOGICAL SOCIETY MEETING HELD AT CALCUTTA ON DEC. 30, 1964

Survival of Piricularia oryzae in soil.—A. APPARAO, College of Agriculture, Rajendranagar, Hyderabad 30 (A. P.)—A study of survival of *P. oryzae* within artificially infected host bits buried in both sterilized and unsterilized soil revealed that the fungus survives for only limited period—less than 19 days—in unsterilized soil, while in sterilized soil it could do so for a slightly longer period. The inability of *P. oryzae* to survive in soil even with the availability of food base suggested that the fungus is unable to stand the intense competition and antagonism of other microflora in soil. Ten actinomycetes, 16 bacteria and 32 fungi were isolated from the rice field soil and were tested for their antagonistic properties to *P. oryzae*. None of the fungi was antagonistic but most of them had overgrown the test fungus. Two strains of *Streptomyces griseus* and one of *S. flaveolus* and five bacteria were strongly antagonistic to the test fungus. The antagonistic properties of the bacteria were due to some principle diffused into the medium.

Effect of vitamins on the growth of Phylloxera-gall and grape stem single-cell clones in tissue culture.—H. C., ARYA, Department of Botany, University of Rajasthan, Jaipur.—Six single-cell clones, 3 isolated from the gall tissue induced by *Phylloxera vastatrix* Planch. and 3 from the normal grape stem tissue were studied on modified White's medium supplemented either with enzyme digest casein hydrolysate (3.0g per litre) or on pure synthetic medium with amino acid combination based on the analysis of casein hydrolysate and alpha-naphthalencacetic acid (NAA, 1.0mg/litre). Effect of different concentrations of i-inositol (75-1250mg/litre), calcium pantothenate (1.0-6.5mg/litre), pyridoxine (1.0—10-O ug/litre), choline chloride (1.0—10.0 ug/litre) and folic acid plus B12 (0.1 plus 0.50—1.0 plus 5 ug/litre) were studied separately. The cultures were adjusted to pH 5.9—6.0 and incubated in the dark at 26°C and around 55 per cent relative humidity. The final readings in wet weights were taken after four weeks of growth. Addition of inositol in the medium increased wet weights of both normal and gall clones with optimum at 300 mg/litre. Increased concentrations of inositol above 300mg/litre reduced the wet weights of all the clones. Calcium pantothenate increased the growth of the normal clones remarkably, in its absence all the three normal clones gave relatively poor growth. The gall clones, however, showed little increase. The maximum growths of the gall and normal clones were obtained with 2.5 mg/litre of calcium pantothenate in the medium. The higher concentrations of calcium pantothenate above 2.5 mg/liter reduced the growths of both the groups of clones.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Addition of pyridoxine and choline into the medium separately in their micro-concentrations did not affect the growth of either gall or normal tissue clones when compared with that on the medium without it. Micro-concentrations of folic acid (Pteroyglutamic acid) plus B_{12} (cyanocobalamin) were used in combination and incorporated in the pure synthetic medium after autoclaving it. The stock solutions of vitamins were sterilized through glass filters and aseptically incorporated in the autoclaved media. Pyridoxine, choline, and calcium pantothenate were eliminated from the medium; Addition of folic acid plus B_{12} increased the growth of both the gall and normal clones. The normal clones showed relatively greater increase in wet weights. 0.25 ug/liter of folic acid plus 0.125 ug/liter of B_{12} in the medium yielded maximum amount of callus in all the tissue clones. With further increase in the concentration of the above vitamins in the medium, the yield of the callus tissue was not reduced significantly in any of the tissue clones. This showed that only a limited amount of the above vitamins in the medium was utilized by the tissues for their metabolism and the rest remained unutilized. But this amount did not induce any toxicity to inhibit growth of the tissues.

Relative susceptibility of brinjal varieties to *Meloidogyne javanica*.—R.B.S. BIRAT, Assistant Plant Protection officer, Bihar, Patna-1. Root-knot nematodes, *Meloidogyne* spp. cause swellings on the roots of susceptible plants. Out of four species, *M. javanica* (Treub, 1885) Chitwood, 1949 is commonly involved in the pathogenicity of a number of vegetable plants in Bihar varying from 58 to 100 per cent. Field observations indicate the incidence of the parasite to the extent of 27.48 per cent on brinjal plants in Patna, Dinapur and Gulzarbagh areas and local variety of brinjal is most susceptible. In micro-field test with nine brinjal varieties, a wide range of variability in respect of varietal susceptibilities was observed.

Relative efficacy of different nematicides for control of root-knot nematode.—R. B. S. BIRAT, Assistant Plant Protection Officer, Bihar, Patna-1. —With a view to screen out suitable soil fumigants for the control of root-knot nematode, a micro-field trial was carried out in randomised block design. The soil fumigants included in the trial were EDB, D-D mixture, ED/CT mixture, formaldehyde (each 20 galls per acre) and Nemagon (2 gallons per acre). Nemagon and D-D provided excellent control for root-knot nematodes. Other fumigants failed to bring about significant results. None of the fumigants exhibited phytotoxic effects at the dosages tried.

Soil treatment with organic insecticides for the control of *Meloidogyne javanica*.—R.B.S. BIRAT, Assistant Plant Protection Officer, Bihar, Patna-1.—Since the use of nematicides is beset with certain limitations, such as high cost, unavailability, toxicity to man and animals, phytotoxicity, etc., it was considered imperative to determine nematicidal properties of some common synthetic organic insecticides as well.

Pot trial with ten insecticides as soil drench and six as dust formulation were carried out. Healthy tomato seedlings were used as indicator plants. It was found that Diazinon 0.2 per cent, Systox

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

0.03 per cent and Metasystox 0.03 per cent provided adequate control to the pest when applied as soil drench at the rate of 150 ml. per pot. Chlordane 5 per cent and Heptachlor 6 per cent when applied as dust formulation at the rate of 1 gm. per pot were assessed superior over D.D.T., B.H.C., Aldrin, Dieldrin and others.

Toximetric studies of Cycloheximide and its five derivatives against onion neck rot organism, Botrytis allii—RAJENDRA K. GROVER, MOHINDER KUMAR, AND SUSHIL KUMAR Department of Botany. Punjab University, Chandigarh and Regional Fruit Experiment Station, Abohar, Punjab.—Cycloheximide (Actidione, Beta. [2 (3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-gluterimide) and its five derivatives, viz., acetate, aceto acetate, methylhydrazone, oxime and semicarbazone, were compared for their toxicity against *Botrytis allii* Munn, the causal agent of onion neck rot. In the spore germination tests cycloheximide was most toxic (ED 50—5.0 ug/ml.) followed by semicarbazone (ED 50—8.0 ug/ml). The dosage-response (DR) curves were parallel for cycloheximide, oxime, and methylhydrazone, while the DR curves for other 3 compounds were parallel to each other. In the poison-food technique tests using Czapek's synthetic agar medium, maximum toxicity was exerted by cycloheximide (ED 50—13.5 ug/ml) and semicarbazone (ED 50—30.0 ug/ml). The DR curves were parallel for cycloheximide, semicarbazone, acetate and oxime, while the curves for acetoacetate and methylhydrazone were parallel among each other. Cycloheximide was most toxic at pH 5.5 and semicarbazone at 5.0. No toxic effect of these chemicals was observed in neutral or alkaline ranges. Cycloheximide and semicarbazone derivative were found to be most effective in controlling the disease. All the chemicals were better protectants than eradicants.

Secretion of protopectinase and polygalacturonase enzymes by Gibberella fujikuroi.—S.C. GUPTA, K. N. Government College, Gyanpur (Varanasi).—*G. fujikuroi* (Saw) Wollenw., a pathogen responsible for wilt diseases of paddy and pea in Almora district, was studied for the secretion of protopectinase (PP) and polygalacturonase (PG) enzymes on natural and synthetic media under different cultural conditions. Decoction media of fruits of tomato and parwal (*Trichosanthes dioica*), tubers of potato and seeds of lucerne (*Medicago sativa*) did not prove to be satisfactory for enzyme secretion. Out of the four synthetic media, all with pectin in them, the one with 0.5 per cent glucose, 1.5 per cent pectin, 0.389 per cent ammonium oxalate, 0.1 per cent potassium dihydrogen phosphate and 0.05 per cent magnesium sulphate was adjuged to be the best for active PP and PG enzyme secretion. It was found that PP and PG both were most active after 7 days of incubation period after which the lysis of the mycelium started and also the activity of the enzyme declined The fungus was found to be adaptive for pectic substances for the secretion of active PP and PG enzymes.

Biochemistry of resistance in cucumber against Cladosporium cucumerinum. II. The role of cucumber phenols on the growth and secretion of pectinolytic enzymes and on pectinolytic enzyme activity.—A. MAHADEVAN, Annamalai. University, Annamalainagar, Madras

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

State.—The scab resistant principle in genetically resistant cucumber plants was attributed to the increased occurrence of phenols in the resistant plants and their subsequent influence on the inhibition of pectinolytic enzyme activity. Following this demonstration, aneffort was made to determine the importance of various cucumber phenols on the growth of the parasite *in vitro* and its enzyme (pectinolytic and cellulolytic) producing ability. The investigation was also extended to determine the extent phenols affected pectinolytic activity. The cucumber phenols employed were caffeic acid, chlorogenic acid, p-hydroxy benzoic acid, p-hydroxy cinnamic acid, ferulic acid and vanillic acid. The phenols were tested at concentrations of 0.1 per cent, 0.5 per cent, and 1 per cent and incororated into a basal Richard's medium. One ml. of spore suspension of the fungus, *C. cucumerinum* was transferred into 250 ml. erlenmeyer flask containing 50 ml. of the final medium with the required concentration and incubated over a four week period, and the results were compared with all the possible combinations of controls.

Almost all the phenols were inhibitory to the growth of the pathogen. However, the toxicity of all the phenols decreased as the incubation period was increased. The decreased toxicity was attributed to lower concentrations of the particular phenol, as determined by the recovery of the phenols, indicating that either the fungus utilized the phenols or converted it into nontoxic by-products However, mono-phenols (p-hydroxy benzoic acid, p-hydroxy cinnamic acid ferulic acid, vanillic acid) tended to show decreased toxicity while di-phenols (Caffeic acid and chlorogenic acid) exhibited slightly more toxicity.

The presence of free pectinolytic enzyme in the culture filtrate was determined viscosimetrically and from the results, it appears that almost all the phenols inhibited the production of the enzyme by the fungus. The nature of cellulolytic activity was not determined. Studies were also conducted to determine the nature of toxicity exhibited by the various culture filtrates containing different phenols on commercial pectinase activity, employing viscosimetric method. It appears that all phenols possessed some inhibitory action on the enzyme and the oxidation products of the phenols also influenced the activity. It is suggested that the defense mechanism in resistant plants should involve the importance of phenolic constituents and their oxidation products on cell wall dissolving enzymes of the parasite.

Studies on soil fungi in sugarcane fields I.—DHIRENDRA PRAKASH AND ABRAR M. KHAN, Botany Department, Muslim University, Aligarh (U. P.).—The soil mycoflora of one year, two-three and three-four years old sugarcane fields has been studied. In all fifteen fungi were isolated. *Stysanus* sp., *Chaetomium* sp., *Thielaviopsis* sp., *Oothecium* sp, *Torula* sp, and *Nigrospora* sp were isolated for the first time from the sugarcane field. There was a build up of saprophytic and parasitic fungi in three-four years old field of sugarcane.

Studies on the Tikka disease of groundnut.—V. RAMAKRISHNA AND A. APPARAO, College of Agriculture, Rajendranagar Hyderabad-30 (A. P.)—The leaf spots of groundnut although caused by two distinct species of *Cercospora*, *C. personata* and *C. arachidicola* are known

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

under one name, *tikka* disease. A detailed study of the symptom picture on seven varieties of groundnut of varied parentage revealed that the colour of the spots on the leaf is a more reliable criterion, than the shape of the lesion and the period of appearance of the halo, in distinguishing the spots caused by the two species of *Cercospora*. The spots on the upper surface are dark brown to tan in the case of both the species where as on the under surface, spots produced by *C. personata* are carbon black and those due to *C. arachidicola* are light brown. A field survey revealed that *C. arachidicola* appears on groundnut 30 days earlier than *C. personata*. due to an early liberation of the inoculum. Humidity was found to have great influence on the spore size, septation and germination and in fection of both the species. A period of 3 days of high humidity was essential for maximum infection in the case of both the species of *Cercospora*.

Production of pectic and cellulolytic enzymes by Rhizoctonia bataticola and Pellicularia praticola.—H. K. SAKSENA AND G. P. SINGH, Department of Plant Pathology, Government Agricultural College, Kanpur.—In an investigation on the role of enzymes in the development of damping off and root rot diseases caused by *Rhizoctonia bataticola* (Taub) Butl. and *Pellicularia praticola* (Kotila) Flentje, the culture filtrates of these fungi were assayed for the presence of polygalacturonase (PG) and cellulase (Cx). *R. bataticola* produced considerable amounts of PG both on sucrose as well as on citrus pectin. *P. practicola* produced only small amounts of PG when citrus pectin was used as a carbon source, and nil or negligible amounts of this enzyme on sucrose containing medium. A weak Cx activity was also detected in the culture filtrates of both *R. bataticola* and *P. praticola*. There were no significant differences in the Cx activity of the culture filtrates of these fungi when grown on different carbon sources. For demonstrating the enzyme production *in vivo*, young seedlings of bhindi were inoculated with *R. bataticola* and those of tomato with *P .praticola*. The production of PG and Cx by *R. bataticola* in culture as well as in the inoculated bhindi seedlings indicates that pectic and cellulolytic enzymes are involved in the pathogenesis by *R. bataticola*.

PG and Cx could not be detected in water extracts from tomato seedlings inoculated with *P. praticola*. *P. praticola* was found to produce PG and Cx in culture although in small amounts only. The symptoms of softening that it produces in the tissues of affected plants, have been attributed to the action of pectic and cellulolytic enzymes in several other diseases It is likely that these enzymes may be active *in vivo* also in case of infection by *P. praticola*, but could not be detected in the water extracts of inoculated tomato seedling possibly due to excessive dilution of the small amounts in which they may be produced *in vivo* by *P. praticola*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological News

PHYTOPATHOLOGIST M. J. Thirumalachar, President of the Society for 1965, has proceeded to U. S. A. to participate and preside over a panel discussion on antifungal antibiotics as a part of the Annual Meeting of the Society of Applied Microbiology, Section of Antibiotics and Chemotherapy at Washington, DC.

CONGRATULATIONS to Geneticist M. S. Swaminathan for receiving Shanti Swarup Memorial Award as well as the Mendel Memorial Medal of the Czechoslovak Academy of Sciences in recognition of his outstanding contributions to genetics.

CONGRATULATIONS to Microbiologist, G. Rangaswami, for his new assignment as the Dean, Agricultural University, Bangalore (Mysore).

PHYTOPATHOLOGIST C. L. Sethi has proceeded on 16th October, 1965 to U. K. for advanced training in Nematology under the Colombo Plan for one year.

ANNOUNCEMENTS

The Annual Meeting of the Indian Phytopathological Society will be held at Chandigarh on January 1 and 2, 1966.

Programme

January 1, 1966 :

9.30 A. M. to 1.00 P. M.—Symposium on rice diseases and their control.

2.30 P. M. to 5.30 P. M.—Symposium on diseases of millets and their control.

7.00 P. M.—Popular lecture.

January 2, 1966

9.00 A. M.—Council meeting.

9.30 A. M. to 1.00 P. M.—Annual meeting of the IPS., Presidential address by Dr. M. J. Thirumalachar, F. N. I., business meeting, opening of ballot papers and announcement of results of election of new office bearers and announcement of Honorary Fellows and introduction of Ordinary Fellows of the Society.

2.30 to 5.30 P. M.—Symposium on maize diseases and their control and group photograph (IPS members).

7.00 P. M.—Third Mundkur Memorial Lecture by Dr. K. D. Bagchee, F. N. I.

8.30 P. M.—Annual dinner (contributory).

Prof. K. S. Thind, Professor of Botany, Punjab University, Chandigarh and Vice-President, I. P. S., may be informed about the accomodation of IPS members, and participation in the annual dinner before December 1, 1965.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

PAGES

Extractability of nucleic acids from the callus tissues of Phylloxera-gall and grape stem—*H. C. Arya* ... 282

Additional viral diseases of temperate fruits in Simla Hills—*B. B. Nagaich and K. S. Vashisth* 288

Studies on the stimulation of conidial germination in Colletotrichum falcatum—*Pritam Singh* 291

Effect of Maleic hydrazide on rusts of Wheat and Barley—*L. M. Joshi* 303

Phytopathological Notes

On the occurrence of Botryosporium palmicolum Speg. in India—*P. N. Mathur and G. Singh* 309

A mosaic disease of runner bean—*K. S. Vashisth and B. B. Nagaich* 311

Helminthosporium rostratum on maize in India—*T. P. Bhowmik and R. Prasada* 312

A mosaic disease of tree tomato (Cyphomandra betacea Sendt.)—*R. D. Joshi and K. S. Bhargava* 313

A new Phyllosticta species on sorghum in India—*D. Suryanarayana and Mahendra Pal* 314

Morus Indica—An additional host of fig mosaic—*K. S. Vashisth and B. B. Nagaich* 315

Tobacco broken ring spot virus from India—*K. S. M. Sastry*... 316

Observations on a mosaic disease of sunflower—*A. N. Battu and H. C. Phatak* 317

Further observations and studies on pigeon pea sterility—*M. L. Seth* 317

A note on aphid transmission of mosaic disease of mulberry—*S. N. Chatterjee and S. P. Raychaudhuri* 319

Abstracts of papers presented at the Indian Phytopathological Society meeting held at Calcutta on Dec. 30, 1964 321

Phytopathological News 326

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00
Cost of Foreign Art Paper with Printing										
2 Pages	3.12	4.25	5.35	6.50	9.75	13.00	16.00	19.00	26.00	32.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1965

President ...	... M. J. THIRUMALACHAR
Immediate Past President ...	... S. SINHA
Vice-President ...	... K. S. THIND
Secretary-Treasurer ...	... S. P. RAYCHAUDHURI (1965-67)
Joint Secretary ...	... M. M. PAYAK (1965-67)

COUNCILLORS

Northern Zone ...	... T. K. NARIANI
Mid-Eastern Zone ...	... B. M. GUPTA
Eastern Zone ...	... S. N. BANNERJEE
Central Zone ...	... B. L. MATHUR
Western Zone ...	... S. P. CAPOOR
Southern Zone ...	... G. RANGASWAMI

EDITORIAL BOARD FOR 1965—67

R. PRASADA
Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU
L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

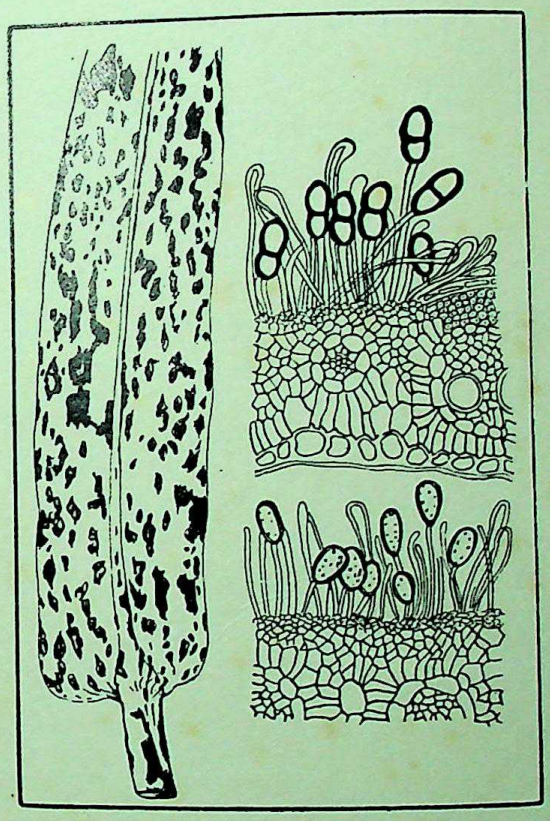
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

VOLUME XVIII 1965 NUMBER 4



PUBLISHED FOR
THE INDIAN PHYTOPATHOLOGICAL SOCIETY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Additions to the fungus flora of Assam-I—*A. K. Roy* ... 327

Metabolites of Fusarium oxysporum f. cumini in relation to cumin wilt—*B. L. Mathur and R. L. Mathur* ... 335

Central Himalayan fungi-II—*S. K. Bose aud Emil Muller* ... 340

Studies on Puccinia hordei II—Formation and germination of teleutospores—*L. M. Joshi* ... 356

The genus Macrolepiota in India—*R. N. Ghosh and N. C. Pathak* 360

Mutation in race 107 of brown rust of wheat Puccinia recondita—*S. K. Sharma and D. P. Misra* 363

Mycostatin for prevention of anthracnose of bananas in storage—*Andal Ananthanarayanan and K. Seshadri* ... 367

Further studies on chirke disease of large cardamom (Amomum subulatum Roxb.)—*S. P. Raychaudhuri and B. Ganguly* 373

Phytopathological Notes

Effect of fungicides on the viability of Brassica seeds—*S. B. Mathur* 378

Effect of zinc and amino acids on the growth of Fusarium oxysporum f. cumini Prasad and Patel—*B. L. Mathur and H. C. Sankhla* 379

Utilization of growth regulators by Fusarium oxysporum f. cumini Prasad and Patel—*H. C. Sankhla, B. L. Mathur and R. L. Mathur* 381

A virulent race of Puccinia striiformis West. on barley and sources for its resistance—*Sheodhan Singh, S. T. Ahmad and D. P. Misra* 382

(Contd. on 3rd cover)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ADDITIONS TO THE FUNGUS FLORA OF ASSAM-I

A. K. Roy

(Accepted for publication November 3, 1965)

Due to high humidity and moderate temperature Assam has an abundant and variegated fungus flora. But little work has been done for their collection and identification. Workers like Butler and Subramanian in 1910-'15, Bose in 1937 and Hooker made some collections from time to time but not in a systematic way. More recently, Chowdhury and Agnihothrudu recorded a good number of fungi including many new spp. and a few genera. This paper deals with describing a new sp., *Periconia shyamala*, together with listing of some fungi which have either been recorded for the first time in India or are new host records for the known species and also the recorded ones from other parts of India but new to Assam. In the former two cases i.e., either new records or new host records for India, brief descriptions have been given while in the last case only the names mentioned. Nos. 9, 10, 12 and 21 are new records and Nos. 8, 11, 15, 20, 26, 28, 34, 36 and 40 are new host records for India.

1. *Ascobolus magnificus* Dodge.

   On cow's dung, Borbheta, 25.7.63, A. K. Roy.

2 *Cercospora abelmoschi* Ellis and Everhart. Subramanian and Ramakrishnan in J. Madras Univ., 22, B : 11, 1952, described it as *C. hibiscina* Ell. and Ev.

   On the leaves of *Abelmoschus esculentus* Moench. (=*Hibiscus esculentus* L.) Karanga, 5.9.63, J Buragohain.

3. *Cercospora canescens* Ellis and Mart.

   On the leaves of *Vigna sinensis* (L.) Endl. ex Haasaka (=*V. catjang* Endl.), Karanga, 4.6. 64, J. Buragohain.

4. *Cercospora gossypina* Cke.

   On the leaves of *Gossypium arboreum* L., Arunachal, 27.12.60, A. K. Roy.

5. *Cercospora grandissima* Rangel.

   On the leaves of *Dahlia* sp., Borbheta, 20.7.63, A. K. Roy.

6. *Chaetomium globosum* Kunze and Schm.

   On decayed paper, Borbheta, 20.6.63, A. K. Roy.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

7. *Chaetomium indicum* Corda.

On decayed paper, Borbheta, 16.6.63, A. K. Roy.

8. *Choanephora cucurbitarum* (Berk. et Rav.) Thaxter.

On the withered petals of *Lilium* sp. Borbheta, 19.7.63, A. K. Roy.

9. *Clitocybe excentrica* Peck., in Bull. Torry Bot. Cl. 1898 : 321.

On dead Stump, Borbheta, 21.6.63, A. K. Roy.

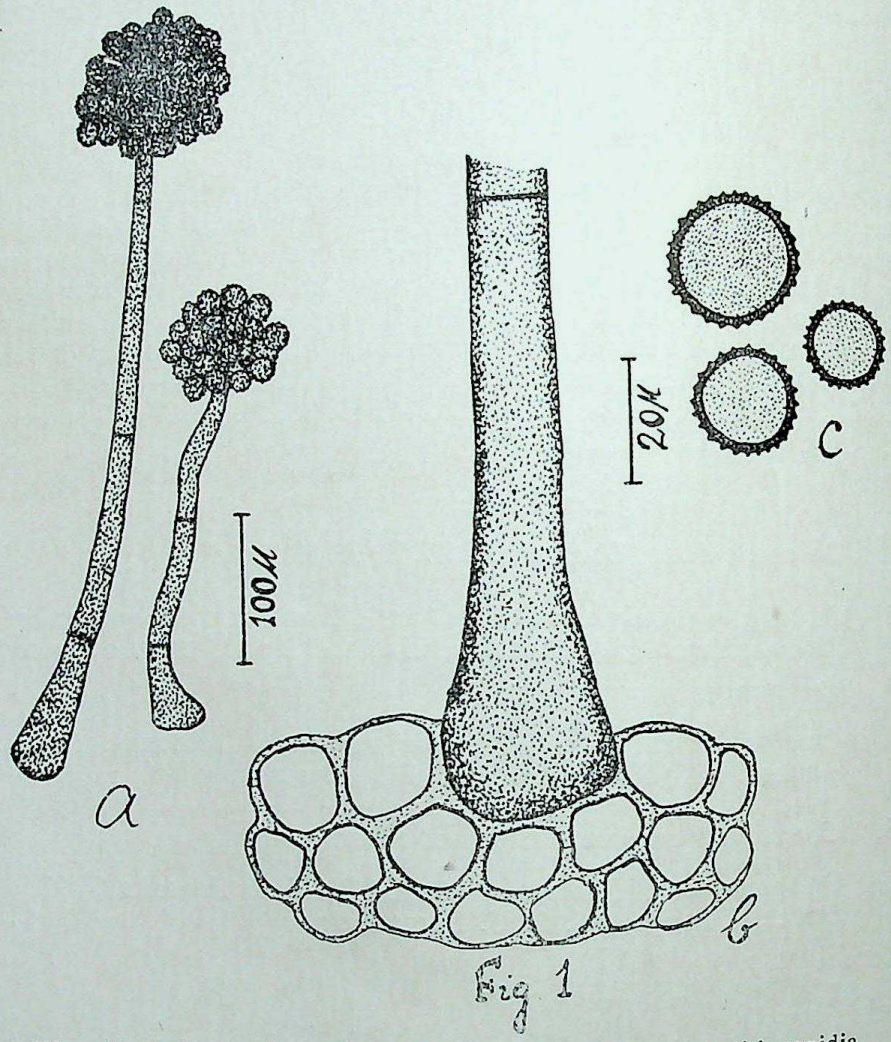


Fig. 1. *Periconia shyamala* spec. nov : a—Contdiophores with conidia, b—base of a conidiophore arising from a stroma, c—conidia.

Sporophores solitary or cespitose ; spore print creamy white. Pileus umbilicate, white, shining, glabrous, often with folds at the periphery, 3.5-6 cm. in diameter, gills typically decurrent. Stipe creamy white, tenacious, neither annulate nor volvate, glabrous at the top and coarsely hairy at the base and measure 3—4.4×0.3—0.5 cm. Spores small, thin walled, elliptic, hyaline, 4—7×1′8—2′5 μ. The conidia are 4—5×2.5—3 μ in the original description (Fig. 4).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

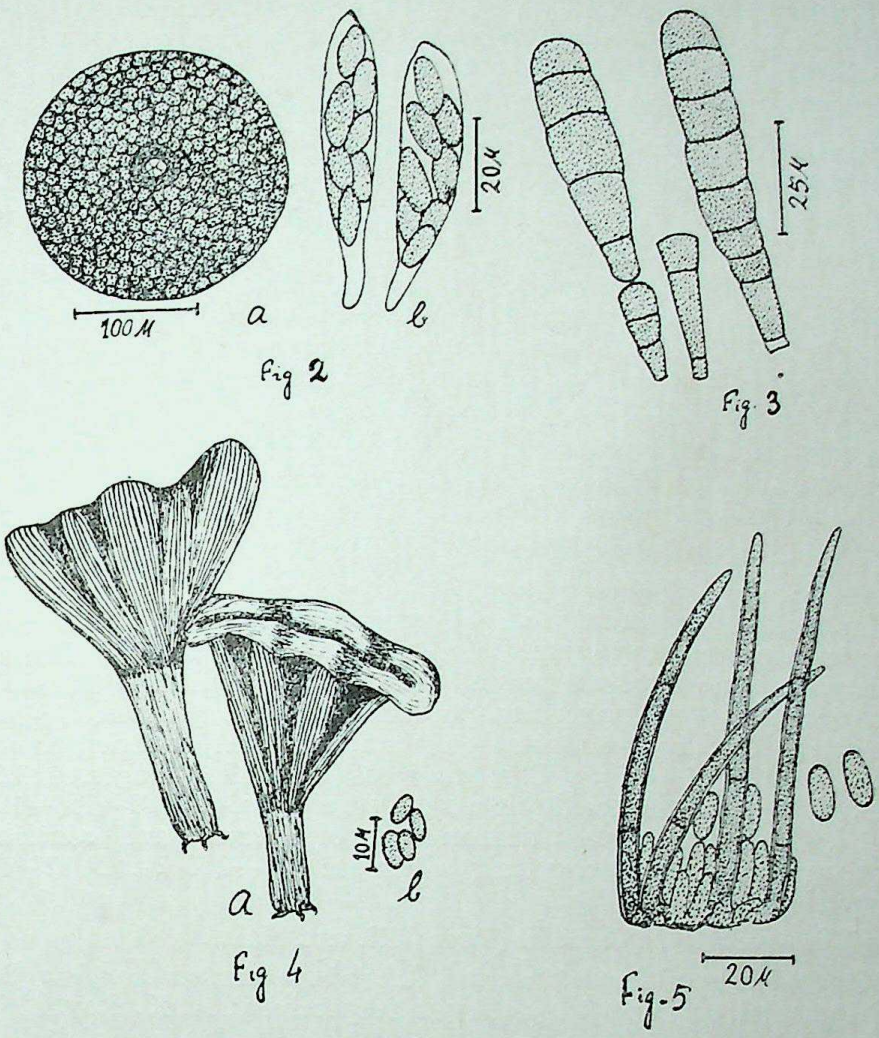


Fig. 2—5. *Guignardia citricarpa* : a—perithecium, b—asci with ascospores. 3. *Stigmina tephrosiae* : Conidia and conidiophores. 4. *Clitocybe excentrica* : a—sporophores, b—spores. 5. *Colletotrichum crotolariae* : a—portion of acervulus, b—conidia.

10. *Cochliobolus cynodontis* Nelson. (Status imperfectus).

On the leaves of *Manihot utilissima* Pohl., Borbheta, 22.12.63, A. K. Roy (H. C. I. O.* No. 28323).

Ocharaceous spots of varying size and shape on the leaves ; colonies hypophyllous, brownish grey. Conidiophores arise singly, light brown to brown, lighter at the tip, straight or slightly bent and the tip may be slightly flexuous with geniculations, 83—138 × 5 μ. Conidia arise terminally, oblong to cylindric with two ends slightly tapering, mostly straight, occasionally slightly bent, 3—9 septate, light brown to dark brown, 33—75 × 11.5—14 μ.

*Herbarium Cryptogamiae Indiae Orientalis, New Delhi, India.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It did not agree either with *Helminthosporium manihotis* Rangel or *H. hispanicola* Cif. reported on the same host earlier but agreed with the conidial stage of the fungus under report.

11. *Colletorichum capsici* (Syd.) Butler and Bisby.

Recently von Arx in Phytopath. Zeit., 29, 1957, has made it a synonym of *C. dematium* (Pers. ex Fr.) Groove.

On the leaves of *Impatiens balsamina* L., Borbheta, 4.6.63, A. K. Roy.

Oblong to linear brown spots on the leaves on which the acervuli develop as minute black, pinhead like bodies. Acervuli punctiform, sub epidermal, 64—121 μ in diameter ; setae brown, lighter at the tip, bristle like, 64—150×3—5 μ. Conidiophores 22—26×2.4—3 μ, conidia 18—22×3.3—4 μ, Butler (1918) described the conidia 17—28×3—4 μ.

12. *Colletotrichum crotalariae* Petch. According to Von Arx in phytopath. Zeit, 29, 1957, it is a synonym of *C. gloeosporioides* Penz. (=*Glomerella cingulata*).

On the leaves of *Inga dulcis* Willd. Borbheta, 30.1.64, A. K. Roy (H. C. I. O. No. 28327).

Brownish spots turning to light ochraceous of varying shape and size, from very small to 18 mm. in diameter on the leaves. Very soon these spots become blighted and fall off. Acervuli epiphyllous, minute, black, dot like, punctiform, discrete, suberumpent, globose, 71-240 μ in diameter. Setae brown, sparsely septate, gradually tapering towards the tip, 40—100×3—4.5 μ ; Conidiophores upto 17 μ long and 3.2 μ broad ; Conidia oblong to elliptic with obtuse ends, (7—) 9—15(—18)× 3.2—4 μ. Here the range of measurement of acervuli and conidia is wider than the original descriptions which are 100—200 μ and 12—16× 5μ respectively. (Fig. 5).

13. *Colletotrichum lagenarium* (Pass.) Ell. and Halst.

On the leaves of *Cucumis sativus* L. and *Benincasa cerifera* Savi, Arunachal, July and August, 1962, respectively, A. K. Roy.

14. *Curvularia lunata* (Wakker) Boedijn.

On incubated leaves and glumes of *Oryza sativa* L., sent from Silchar, 2.8.63.

15. *Corynespora cassiicola* (Berk. and Curt.) Wei, in. Mohanty and Mohanty, 1955, *Sci. and Cult.*, 21 : 330—332 ; Mohanty, 1958, *Sci. and Cult.*, 23, 608-609; Ramakrishnan, 1950, *Proc. Indian Acad. Sci.*, B, 51 : 164—168 ; Agarwal, 1961, *J. Indian Bot. Soc.*, 40, 404—408 : Agnihothrudu, 1962, *J. Indian Bot. Soc*,, 41 : 475.

On the leaves of *Manihot ultilissima* Pohl., Borbheta, 14.11.63, A. K. Roy, (H. C. I. O. No. 28246).

Brown diffuse leaf spots of variable size and shape, upto 2.5 cm. diameter. Conidiophores hypophyllous, forming a greyish colony with the production conidia, arise singly, 3—7 septate, light brown to brown with 0—7 apical proliferations which are lighter coloured, the bottom end in a bulbous cell, measure 136—350×5.5—8 μ. Conidia arise singly or seldom in chain, cylindro-clavate with truncate base, pale olivaceous brown, 4—14 pseudo septate, 75—151×14.5—21 μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

16. *Cyathus striatus* (Huds) Hoffm.,

One dead stump, Silchar, June, 1961, A. K. Roy.

17. *Diplocarpon rosae* wolf.

On the leaves of *Rosa multiflora* Thumb, Karanga., 27.2.64 J. Buragohain.

18. *Fusicladium pongamiae* Sydow.

On the leaves of *Pongamia glabra* Vent. (=*P. pinnata* Merr.), Karanga, 3.6.64, J. Buragohain.

19. *Gloeosporium palmarum* Oudem.

On the fruits of *Areca catechu* L., Madhabpur, 4.3.64, A. K. Roy.

20. *Glomerella cingulata* (Stonem.) Spaulding and Von Schrenk.

On the leaves of *Pandanus minor* P. Henn., Borbheta, 24.6.63. A. K. Roy.

Oblong to liner brown spots on the leaves, finally drying up. Asci clavate cylindric, closely packed inside the perithecia, 42—62×6—9 μ; ascospores hyaline, elliptic, sometimes slightly bent and narrower with obtuse ends, generally with one vacuole, 11—15×4—5 μ. Von Arx and Muller (1954) described the asci 35—80×8—14 μ and ascospores 9—30×3—8 μ.

21. *Guignardia citricarapa* Kiely, in J. Aust. Inst. Agric. Sci. 1948, 14, 81—83 ; Agric. Gaz. N. S. W., 1949, 60 ; 17-29 ; Schuep 1961, phytopath. Z., 40 : 258—271.

On the leaves of *Dioscorea pentaphylla* L., Tipomia, 25.9.63, J. Buragohain (H. C. I. O. No. 28247).

Dark brown circular spots 2—10 mm. diameter on the leaves, centre becoming grey with the advance of age on which minute, black, pinhead like perithecia develop. Perithecia amphigenous but mostly epiphyllous and rarely hypophyllous, flask shaped, ostiolate, brown, cellular, 58—135 μ in diameter. Asci bitunicate, obclavate, 44—78×9—12 μ ; ascospores ovate to elliptic, hyaline, 10—14×4.8—6.5 μ (Fig. 2).

22. *Helminthosporium frumentaceum* Mitra.**

On the leaves of *Echnichloa frumentaceum* Munro. (=*Panicum frumentaceum* Roxb.), Karanga, 4.6.64, J. Buragohain.

23. *Meliola jasminicola* P. Henn.

On the leaves of *Jasminum sambac* Ait., Karanga. 4.9.63, J. Buragohain.

24. *Meliola psidi* Fr.

On the leaves of *Psidium guajava* L., Borbheta, 2.11.63, M. Hazarika.

**Though redesignated as *Bipolaris*, *Helminthosporium* is retained here which is commonly used.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

25. *Oidium cyparissiae* Syd.

On the leaves of *Euphorbia hirtha* L., Arunachal, February' 61, A. K. Roy.

26 *Oidium erysiphoides* Fr., in Sacc., 1886, Syll. Fung., 4 : 41 Mitter and Tandon : 1930, J. Indian Bot. Soc. 9 : 197.

On the leaves of *Impatiens balsamina* L. and *Dahlia* sp. Borbheta, 27.6.63 and 11.2.64, respectively, A. K. Roy (H. C. I. O. No. 28328 on *Dahlia*).

Circular white chalky spots on the upper surface of the leaves, gradually covering whole of them causing drying and falling off. Mycelium sparingly branched and septate, 5—10 μ thick ; conidiophores long, septate, gradually broadening towards the tip ; conidia hyaline to sub-hyaline granular, oval to elliptic, measuring 27—45 × 16—26 μ in case of *Impatiens* and 10—30 × 16—19 μ in *Dahlia*, In the original description the conidia are 30—40 × 15—20 μ.

27. *Periconia shyamala* A. K. Roy, sp. nov.

Maculae ochraceae ad 15 mm. diam. in foliis ; coloniae hypopyllae, effusae, nigrae, pilosae. Stroma irregularis formae, pseudoparenchymaticum, pallide brunneum ad 65 μ latum ; conidiphore macronemata emergunt e stromate singulariter vel rarius bina, vulgo recta, interdum flexuosa, robusta, brunnea, sparse septata (vulgo biseptata), septo basali viciniori, basi paulum tumescente, 168—432 × 12—17 μ. cellulae sporogenae evolvuntur ad apicem conidiophori. ex ovoideis subsphaericae, pallide brunneae, parietibus tenuibus praeditae, conidia supportantes. Conidia globosa, brunnea, echinulata, 16—22 μ diam., episporio 1'5 μ crasso.

In foliis *Manihot utillissimae* Pohl, ad Borbheta in Assamia, 22.12.1963, A. K. Roy, posita in hurb. Crypt. Indiae Orientalis ad New Delhi sub numero 28355.

Ochraceous spots upto 15 mm. diameter on the leaves ; colonies hypophyllous, effused, black, hairy. Stroma irregular in shape, psuedoparenchymatous, light brown upto 65 μ wide ; macronematous conidiophores arise from the stroma singly or rarely in two, mostly straight or occasionally flexuous, stout, brown sparcely esptate (generally bi—), the basal septum is closer, base slightly bulbous, 168—432 × 12—17 μ. Sporogenous cells develop at the tip of conidiophore, ovoid to sub-sphaerical, light brown, thin walled and bear the conidia. Conidia globose, brown, echinulate, 16—22 μ in diameter, epispore 1.5 μ thick. (Fig. 1).

The measurement of conidia lies in between *Periconia byssoides* (12—14 μ diam.) and *P. manihoticola* (=*P. heveae*) (25—30 μ diam.).

28. *Pestalotia albo-maculans* P. Henn., (sensu Guba).
On the leaves of Inga dulcis Willd., Borbheta, 17-7-63, A. K. Roy, (H. C. I. O. No. 28248).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The description has been given elsewhere.

29. *Pestalotia piperis* Petch (sensu Guba).

On the leaves of *Piper nigrum* L., Buralikson, 10.3.64, A. K. Roy.

30. *Pestalotiopsis mangiferae* (P. Henn.) Steyaert. Sarkar in Lloydia, 1960, 23 : 1—7, has described it as *Pestalotia mangiferae* Butl.

On the leaves of *Mangifera indica* L., Arunachal, July, 1961, A. K. Roy.

31. *Phyllosticta ingae-dulcis* Died.

On the leaves of *Inga dulcis* Willd., Borbheta, 17.2.64, A. K. Roy.

32. *Phyllosticta miurai* I. Miyake.

On the incubated leaf sheath and culm of *Oryza sativa* L., sent from Silchar, 2.8.63.

33. *Phyllostictina artocarpina* (Syd. and Butl.) Syd.

On the leaves of *Artocarpus intergifolia* L. (=*A. heterophyllus* Lam.) Tipomia, 21.4.64, J. Buragohain.

34. *Piricularia grisea* (cke.) Sacc., in Padwaick, 1950, Manual of Rice diseases : 9.

On the leaves of *Digitaria sanguinalis* (L.) Scop., Borbheta, 9.7.63, A. K. Roy (H. C. I. O. No. 28251).

Small and few, more or less oval purplish spots becoming brown limited by purplish margin 2—6×1—3 mm. in size, on the leaves. Conidiophores arise in fascicle, simple, sparingly septate, olivaceous to light fuligenous, 55—114×4—6.5 μ ; Conidia obclavate, tapering at the apex, extending to a short tooth at the base, 3 or rarely 2 celled, sub-hyaline to pale olive, 16—32×6—8 μ.

35. *Phyllochora cynodontis* (Sacc.) Niessl.

On the leaves of *Cynodon dactylon* Pers., Borbheta, 20.5.64, A. K. Roy.

36. Phyllachora graminis (Pers.) Fuck., in Butler and Bisby, Sci. Mono. 1, I. C. A. R., 1931 : 34.

On the leaves of *Cyperus rotundus* L., Nagajanka, 5.9.63, J. Buragohain.

Amphigenous black spots upto 3 mm. long, on the leaves. Perithecia gregarious in a row in the stroma, 57—178 μ in diameter. Asci clavate-cylindric, 68—90×6.5—8 μ ; ascospores elliptic, slightly tapering with obtuse ends, guttulate, 12—17×3.2—4.8 μ. In the original description the asci are 78—80×7—8 μ and ascospores 8—12×4—5 μ.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

37. *Pseudoperonospora cubensis* (Berk and Cutr.) Rostow, Uppal et al. in Bull No. 176, Dept of Agri. Bombay, 1934 : 2, described it as *Peronoplasmopara cubensis* (Berlese) Rost.

On the leaves of *Cucumis sativus* L., Jorhat, 29.5.63, T. Neog.

38. *Puccinia romagnoliana* Maire and Sacc.

On the leaves of *Cyperus rotundus* L., Nagajanka. 11.9.63, J. Buragohain. Only the uredial stage found.

39. *Ravenelia sessilis* Berk.

On the leaves of *Albizzia lebbk* Benth., Arunachal, 26.12.60, A. K. Roy. Only the uredial stage observed.

40. *Stigmina tephrosiae* Agnihothrudu, in J. Indian Bot. Soc., 41 (3) : 459—464, 1962.

On the leaves of *Boga medeloa*, Tinsukia, 13.3.64, A. K. Roy (H. C. I. O. No. 28249).

Spots more or less globose, brown, well demarcated, upto 3 mm. in diameter, solitary or rarely coalescing, on the leaves. Sporodochia hypophyllous, erumpent, minute, discrete, almost black, 47—92 μ in diameter. Conidiophores erumpent, radiating straight, gradually broader at the apex, olivaceous brown to yellowish brown, generally 2—3 septate, measuring $28-48 \times 10-14$ μ. Conidia arise singly at the apex as blown out ends, cylindro-clavate, olivaceous brown, 3—8 septate, consitricted at the septa, truncate at the base with a collar of conidiophore, 44—126 $(-140) \times 17-26(-29)$ μ in size. Agnihothrudu (1962) described the sporodochia upto 120 μ diam., conidiophores upto 40 μ long and $8-10$ μ wide, conidia 3—5 septate, $40-90 \times 12-22$ μ (Fig. 3).

41. *Uncinula necator* (Schow.) Burr.

On the leaves of *Vitis vinifera* L., Borbheta, 22.6.63, A. K. Roy.

42. *Uromyces appendiculatus* (Pers.) Unger.

On the leaves of *Phaseolus vulgaris* L., Arunachal, 27.2.63, A. K. Roy.

ACKNOWLEDGEMENT : My thanks are due to Rev. Fr. Dr. H. Santapau, Director, Botanical Survey of India, for rendering latin diagnosis of the new species and to Dr. Ellis, Dr. Booth, Mr. Sutton of the Commonwealth Mycological Institute, London, and Dr. V. Agnihothrudu, Coffee Research Unit, Bangalore, for their kind help in identifying some of the spp. The services rendered by the staff of the Mycology Division are also gratefully acknowledged.

Division of Mycology,
Department of Agriculture, Assam,
Jorhat.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

METABOLITES OF FUSARIUM OXYSPORUM F. CUMINI IN RELATION TO CUMIN WILT

B. L. MATHUR AND R. L. MATHUR

(Accepted for publication November, 12, 1965)

Wilt inducing pathogens have long been thought to produce toxic metabolites which are liberated into the host and transloçated to leaf cells, destroying their differential permeability and thus causing wilt. The work on the toxin production has been reviewed by Sadasivan (1959) and Wheeler and Luke (1963). Amongst wilt disease toxins, the most widely worked out problem is that of tomato wilt caused by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. and Han. The metabolites produce characteristic drooping of leaves, flagging of petiole along with vascular browning and finally wilting. The metabolites of wilt *Fusaria* were found to be non-specific, dialyzable and thermostable (Sadasivan, 1958 ; Venkata Ram, 1957). The present study represents an attempt to assess the effectiveness of the metabolites from *Fusarium oxysporum* f. *cumini* Prasad and Patel.

MATERIAL AND METHODS : In these studies a highly pathogenic isolate of *Fusarium oxysporum* f. *cumini* was used (Mathur and Prasad, 1964). Other vascular pathogens included were the organisms causing wilts of coriander, linseed, pea, lentil, gram, and tobacco. The cultures were grown in Richard's solution in 500 ml. flasks under static conditions at room temperature for 20 days. The fungal mat was filtered through Chamberlain filters and the filtrate was tested for the production of toxin. To examine the influence of age of culture on toxin production the fungi were grown for different lengths of time and the respective filtrates were tested for their toxicity. The filtrate obtained from twenty day old cultures was sterilized to examine the effect of high temperature on the toxicity of the filtrates. The retention of toxicity on dilution was studied by diluting the filtrate with sterilized double distilled water in different proportions.

One month old plants grown in sterilized soil were used. The plants in each test were carefully selected for uniformity and the roots were thoroughly washed with distilled water. Sufficient quantity of filtrate was added in the tubes and the plants inserted into the tubes so as to cover root portion only. The time taken for the wilt symptoms to appear was noted in each treatment.

RESULTS : *Effect of cumin-wilt culture filtrate on seedlings* : Time taken for wilting of either seedlings with intact roots or for cut shoots was the same. The seedlings began to wilt after 20 hours in both the treatments, in 48 hours the seedlings dried up and the leaves and the stem became brittle.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The seedlings of *Coriandrum sativum* L., *Daucus carota* L., *Foeniculum vulgare* Gaertu, *Anethum graveolens* B and H, *Carum copticum* B and H, *Lens esculenta* Moench, *Linum usitatissimum* L., *Cicer arietinum* L., on treatment with culture filtrate showed tip injury to the leaves and wilted in 20 hours, showing flagging of petioles and drooping of stem. The plants were green till complete drying took place. In *Pisum sativum* L., and *Lycopersicum esculentum* Mill. seedlings, leaf blade injury and water-soaked spots on the margins of the leaf-lets were visible in 24 hours. The flagging of petioles started within 30 hours and complete wilting took place in 46 hours. No softening of the root portion was observed ; browning of root and stem was also absent.

Production of wilt in cumin by metabolites from other pathogens : The filtrates from *Fusarium oxysporum* f. *coriandrii* (Kulkarni, Nikam and Joshi) *F. oxysporum* f. *lini* (Bolley) Snyd. and Han., *F. oxysporum* f. *pisi* (Lind.) Snyd. and Han., *F. oxysporum* f. *nicotianae* Johnson, *F. oxysporum* f. *ciceri* (Padwick) Snyd. and Han., *F. orthoceras* f. *lentis* (Vasudeva and Srin.) incited wilt in cumin plants. In one month old seedlings, leaves showed drooping symptoms followed by flagging of petiole and stem folding. The plants dried within 48 hours.

Effect of period of growth of fungus on wilt-inducing capacity : The production of toxic symptoms were directly related with the period of growth of the fungus in culture (Table 1). The seedlings wilted after 48 hours in 12 day old culture filtrate but 30 day old culture filtrate took 12 hours to induce wilting. The period of wilting in 18 day old culture filtrate was 20 hours and 16 hours in 24 day old culture filtrate.

TABLE 1 : Effect of period of growth on toxin production.

Period of growth in days	Time taken in hours for wilting
12	48
18	20
24	16
30	12
Control (Water only)	All healthy

Effect of boiling and sterilization of culture filtrates on wilt-inducing capacity : Unboiled filtrates produced drooping of leaves, then flagging of petioles and ultimately followed by wilting in 20 hours. The leaf blade injury was equally severe with heated and unheated filtrates and yellowing was not observed in any of the treatments. The toxin in filtrate was not destroyed either by boiling, or heating for 15 minutes at 60° C or by sterilization at 6 lb. pressure for 10 minutes, although the effect of toxin was reduced to some extent (Table 2).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Effect of boiling and sterilization of 20 days old culture filtrate.

Different treatments of filtrates	Time taken in hours for wilting
Unboiled	20
Heated at 60°C. for 15 minutes	30
Boiled for 5 minutes	46
Sterilized at 6 lb. pressure for 10 minutes	46
Control (Water only)	No wilting

Effect of dilution af culture filtrate on wilting : The time taken for symptom production was directly related to dilution ; more diluted the solution, longer the time taken to produce the symptoms of wilt (Table 3). The browning symptoms were not visible even when the plants were kept in dilute filtrate for more than 3 days as shown by Scheffer and Walker (1953). The browning entity was not produced in this culture filtrate up to 30 days of growth.

TABLE 3 : Effect of dilution of culture filtrate on wilting.

Proportion of filtrate and water	Time taken in hours for wilting
6 : 0	20
5 : 1	30
4 : 2	46
3 : 3	46
2 : 4	70
1 : 5	80
Control (Water only)	No wilting

DISCUSSION : The culture filtrate of *Fusarium oxysporum* f. *cumini* produced wilting symptoms in coriander, carrot, dill, fennel, carum, lentil, linseed, gram, pea and tomato. The symptoms of wilting such as peculiar drooping of the leaves and flagging of the petioles were observed in all the host plants. Leaf blade injury and water-soaked spots were visible in pea and tomato only. Vascular browning was not observed in any host

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

plant. The filtrates prepared from cultures of *Fusarium oxysporum* f. *coriandrii*, *F. oxysporum* f. *lini*, *F. oxysporum* f. *pisi*, *F. oxysporum* f. *nicotianae*, *F. oxysporum* f. *ciceri*, and *Fusarium orthoceras* f. *lentis* were capable of inducing wilt in cumin plants. Winstead and Walker (1954 a) reported that *Fusarium* wilt organism inciting tomato wilt, cotton wilt, cabbage yellows, pea wilt, and pea near-wilt induced vascular browning in all plants tested. The browning factor was common to all the wilt organisms and was not specific for the respective susceptible host. Winstead and Walker (1954 b) working on cabbage, radish and cotton wilt *Fusaria* showed that metabolites in culture from these pathogens were specific in their effect, and that culture fluids caused typical external symptoms on susceptible varieties but did not cause vascular browning.

Winstead and Walker (1954 a) further reported that heating *Fusarium* culture filtrates for 10 minutes at temperatures above 60°C. greatly reduced their activity. Chi and Hansen (1964) reported that heat greatly reduced the activity of culture filtrate from red clover *Fusaria* but did not destroy it. Some activity was observed even when the filtrate was heated to 80°C for 20 minutes. In the present tests with filtrate from cumin wilt *Fusarium*, the metabolites retain their effect even when boiled but under steam pressure its toxicity was slightly reduced. This is in line with the observations of Naim (1958) on cotton wilt. The metabolites produced by cumin wilt *Fasarium* withstand dilution in double distilled water. The toxicity being directly proportional to the concentration of the filtrate, a highly diluted solution takes much longer time to produce wilting than a concentrated solution. Similar observations have been recorded by Chauhan (1961) on gram wilt *Fusarium*.

Summary

The metabolites produced by *Fusarium oxysporum* f. *cumini* induced wilting symptoms in coriander, carrot, carum, fennel, dill, lentil, linseed, gram, tomato and pea. The filtrate retained its effect after sterilization for 10 minutes at 6 lb. pressure and on dilution up to 6 times. No browning symptoms were observed in various hosts tested with the toxin.

Acknowledgement : Thanks are due to the Director of Agriculture, Rajasthan, Jaipur for providing laboratory facilities.

Plant Pathology Laboratory,
Rajasthan, Jaipur.

References

Chauhan, S. K. (1961) Toxin production by *Fusarium orthoceras* var. *ciceri* causing wilt of gram. *Proc. Nat. Acad. Sci.* **31** : 341-48.

Chi, C. C. and E. W. Hanson (1964) Mechanism of wilting incited by *Fusarium* in red clover. *Phytopathology* **54** : 646-653.

Mathur, B. L. and N. Prasad (1964) Studies on wilt disease of cumin caused by *Fusarium oxysporum* f. *cumini*. *Indian J. agric. Sci.* **34** : 131-137.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

NAIM, M. S. (1958) Responses of wilt resistance aud susceptible cotton variety cut shoots to metabolites of different *Fusarium* sp. *A' in Shame. Sci. Bull.* **2** : 45-54.

SADASIVAN, T. S. (1958) Presidential address : Moulds, Metabolites and tissues. *Sec. Bot. 45th Proc. Indian Sci. Cong. Madras.*

SADASIVAN, T. S. (1959) Recent advances in Botany. 2 : 1021-24. *Univ.* of *Torento Press, Torento.*

SCHEFFER, R. P. AND J. C. WALKER (1953) The physiology of *Fusarium* wilt of tomato. *Phytopathology* **43** : 116-25.

VENKATA RAM, C. S. (1957) Fusaric acid production by *Fusarium orthoceras* in vitro. *Experientia* **13** : 284.

WHEELER, H. AND H. H. LUKE (1963) Microbial toxins in Plant Diseases *Ann. Rev. Microbiol.* **17** : 223-42.

WINSTEAD, N. N. AND J. C. WALKER (1954 a) Production of vascular browning by metabolites from several pathogens. *Phytopathology* **44** : 153-58.

WINSTEAD, N. N. AND J. C. WALKER (1954 b) Toxic metabolites of the pathogen in relation to *Fusarium* resistance. *Phytopathology* **44** : 159-166.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CENTRAL HIMALAYAN FUNGI-II

S. K. Bose and Emil Müller

(Accepted for publication July 12, 1965)

In the first part of this series, the authors had described a number of fungi, mostly occurring on various species of *Quercus*. The present paper comprises of the result of studies conducted on fungi found on a wider host range. The species described here and the related figures have been numbered in continuation of those given in the first part (Bose and Müller, 1964). The collections, including the type specimens, have been deposited in the ETH Herbarium, Zürich, Switzerland.

15. *Lophodermium querci* nov. spec.

Ascomata epiphylla, dispersa, subcuticula nascentia, lenticulatia vel triangulata, 400—600 μ longitudine, 150—170 μ latitudine et 200—350 μ altitudine. Paries laterae apicaliter 40—50 μ, lateraliter 15—22 μ crassitudine. Fissum linerum ad 40 μ apertum, labris incrassatis, hyphis hyalinis, septis compositis circumdatum. Asci longe stipitati, pars spor. cylindracei, 70—125 × 8—12 μ, 8—spori. Sporae clavato-filiformae, 5—10—septatae, 40—50 × 1.5—2 μ, hyalinae. Paraphyses filiformes, 1—1.5 μ crassitudine, apice uncinates.

Status conidiophorus : *Leptostroma*.

Hab. in foliis vivis *Quercus leucotrichophori* A. Camus, India, Kumaon, Chaubattia (Ranikhet) September 18, 1962, leg. T. C. Sharma.

The subcuticular, lens-shaped or triangular ascocarps are scattered on the upper surface of living leaves of *Quercus leucotrichophora* A. Camus, and measure 400—600 × 150—170 μ. The wall of the ascocarp is 40—50 μ in thickness towards the ostiole, gradually becoming thinner at the sides where it is 15—22 μ in thickness and is composed of dark brown, polyhedral cells, measuring 2—6 μ in diameter. Towards the ostiole, the cells become elongated, septate, hyaline and 5—16 μ in length. The flat base of the ascocarp is composed of two or three layers of elongated or polyhedral, brown cells. The ostiole is linear and upto 40 μ in width (fig. 15).

The asci are unitunicate, elongated club-shaped with a long stipe and measure 70—125 × 8—12 μ. The apices of the mature and immature asci are at different levels and point towards the labia. The ascospores are hyaline, cylindrical-clavate, multiseptate (usually 5—10 septate), parallel and 40—50 × 1.5—2 μ in size.

In the rearrangement of the genus *Lophodermium*, Tehon (1935) has reported *Lophodermium punctiforme* (Fr.) Fuckel to occur in Europe

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and north America on the leaves of various species of *Quercus* and allied hosts. The *Lophodermium* on *Q. leucotrichophora*, differs from this species particularly in having septate ascospores. In this characteristic it also differs from most of the other species of *Lophodermium*. Only *L. septatum* (Tehon) Terrier (1942), occurring on *Picea sitchensis* (Bongarde) Carr. in north west region of the United States, also has septate ascospores, but these are double the length of the ascospores of the species described above. In other characters, *L. querci* agrees with other species of the genus and it is also associated with an imperfect stage, belonging to *Leptostroma*.

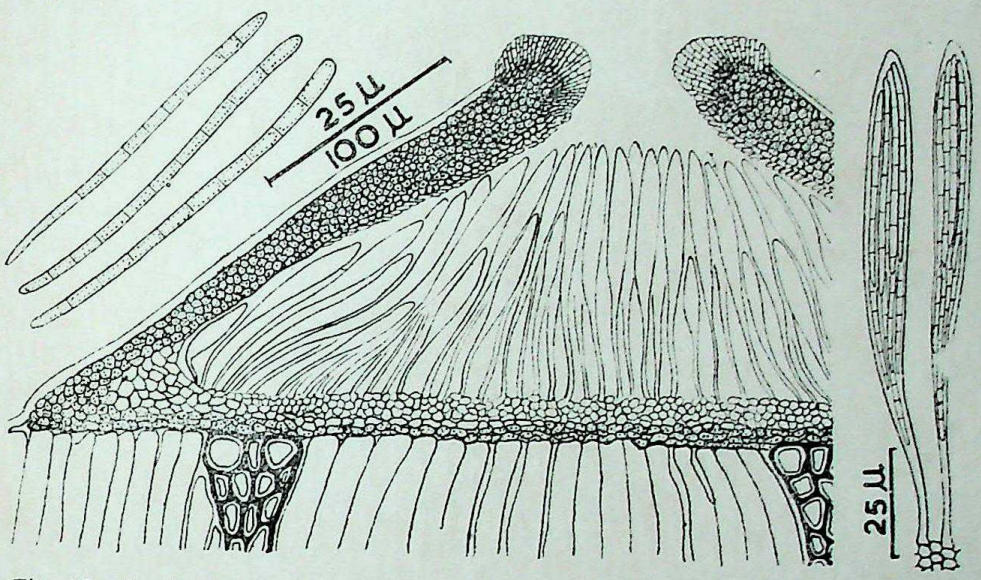


Fig. 15. *Lophodermium querci*. Section through an ascocarp, 250x. Left—Ascospores, 1,000x. Right—Asci, 500x.

16. *Acantharia quercus-dilatatae* nov. spec.

Hypostroma subcuticulare, 15—32 μ crassitudine, cellulis hyalinis, tenuiter tunicatis compositum et stromatibus erumpentibus, pulvinatis, 150—190 μ diam. portante. Perithecia globosa vel subglobosa 350—425 μ altitudine, 350—510 μ diam. Paries peritheciorum 55—95 μ crassitudine, cellulis polyedricis, fuscis, 12—22 μ diam. compositus. Ostiolum planum, poro rotundato, ad 45 μ diam. apertum. Asci bitunicati, cylindracei, 112—150 × 12—22.5 μ, 8—spori. Sporae ellipsoideae vel clavatae, supra medium septatae et constrictae, 19—28 × 9—13.5 μ, cellulis ultimis incrassatae.

Hab. in foliis vivis *Quercus dilatatae* Lindl., India, Kumaon, Gwaldam (Chamoli), April 30, 1964, leg. B. S. Khati.

The fungus occurs on living leaves of *Quercus dilatata* and forms circular, brownish-black spots, growing upto 12 mm. in diameter. The spots may coalesce to form larger patches. Within the leaf, the fungus forms a subcuticular hypostroma, 15—32 μ in thickness and composed of several tiers of thin-walled, hyaline, vacuolated, cubical cells, measuring 4—8 μ in width. On the surface, cushion-shaped stromatic structures are formed. These are 150—900 μ in diameter and are composed of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

thick-walled, dark brown, cubical cells measuring 4—12 μ in height. The cells on the surface of the stroma elongate into thick-walled, encrusted or warty, blunt bristles, upto 28 μ in length and 6—10 μ in width. In early stages, these bear spherical, warty, conidia-like cells, measuring upto 13 μ in diameter (fig. 16).

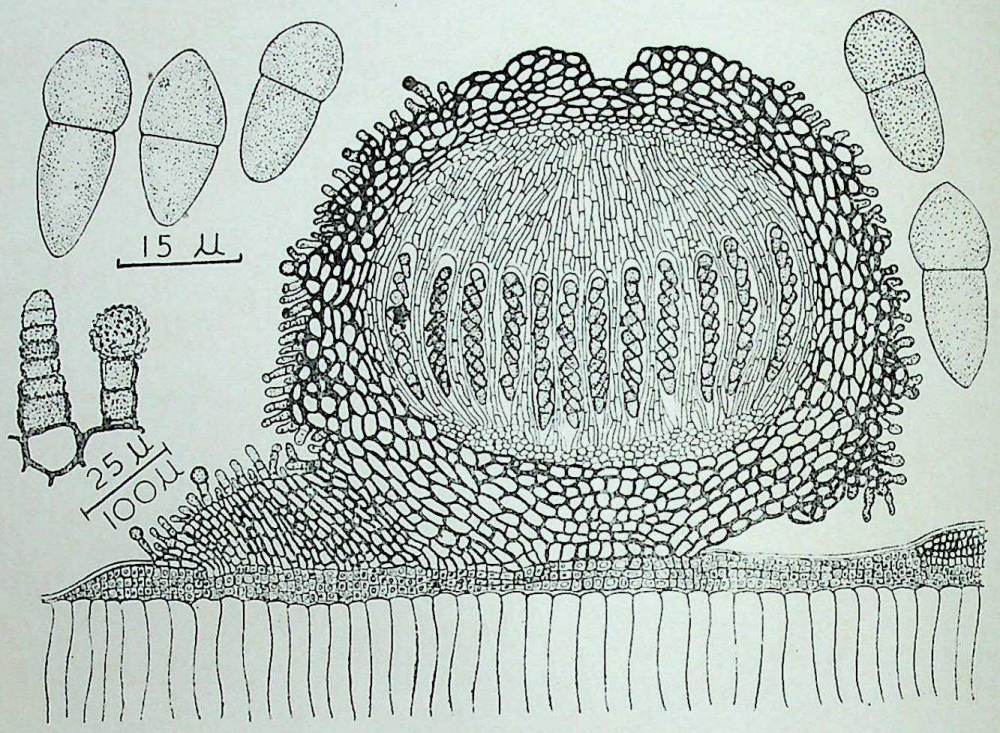


Fig. 16. *Acantharia quercus-dilatatae*. Section through an ascocarp, 125x. Above—Peripheral cells of stroma with bristles, bearing conidia-like cells, 500x. Ascospores, 1000x.

The ascocarps arise from the stromata and are spherical or usually slightly flattened at the apex. In early stages, these are also beset with the blunt bristles which cut off warty, conidia-like cells. The ascocarps are 350—425 μ in height and 350—510 μ in diameter. The wall of the ascocarp is 55—90 μ in thickness at the apex and sides, becoming somewhat thicker at the base, and is composed of brown polyhedral cells, measuring 12—22 μ in diameter. The mature ascocarp opens by means of a schizogonous pore which is upto 45 μ in diameter.

The asci are cylindrical, truncate at the base, rounded and thick-walled at the apex and measure 112—150 × 15—22.5 μ. The eight ascospores are biseriate in the beginning, becoming overlappingly uniseriate at maturity. These are broadly ellipsoid, rounded or pointed at one or both ends, bi-celled, constricted at the septa and 19—28 × 9—13.5 μ in size. The upper cell is broader and usually shorter than the lower cell. A few ascospores have been observed to be septate in the middle.

The fungus, differs from the other species of *Acantharia* as the ascospores are usually septate above the middle. The structure of the ascocarp, stroma and stromatic bristles, reminds one of *A. sinensis* (Petr.) v. Arx (1958), the ascospores of which are septate in the middle.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Müller (1958) has recorded the occurrence of this fungus in the central Himalayas, where it is a parasite on *Castanopsis indica* A. D. C.

17. *Massarina parasitica* nov. spec.

Perithecia plus minusve hemisphaerica, 175—275 μ diam. et 100—125 μ altitudine, ostiolo rotundato, 8—15 μ crassitudine aperta, clypeo atro-brunneo instrata. Paries peritheciorum apicaliter 15—25 μ, lateraliter 10—15 μ crassitudine, cellulis brunneis, polyedricis, 4 μ diam. compositus. Asci bitunicati, cylindraceo-clavati, 40—65 × 8—11 μ, 8—spori. Ascosporae biseriatae, fusiformes, transverse 1—3—septatae, hyalinae, 12—18 × 2.5—4.5 μ. Paraphysoides hyalinae, fibbrosae.

Status pycnidiophorus : *Stagonospora.*

Hab. in foliis viventibus *Micheliae duthii* King. (*Lauraceae*), associatis *Cephaleuri parasitici* Karst. India, Kumaon, Chaubttia, (Ranikhet), July 27, 1962, leg. A. J. Roy.

The fungus occurs on the upper surface of the living leaves of *Michelus duthie* in spots affected with the algal parasite, *Cephaleuros parasiticus*. The ascocarps are subepidermal 175—275 × 100—125 μ in size hemispherical, and are surrounded by the algal thallus. The wall of the ascocarp is 15—25 μ in thickness at the apex and composed of dark brown, polyhedral cells upto 4 μ in diameter. These cells also ramify intercellularly within the epidermis, forming a clypeus. The wall of the ascocarp is 10—15 μ in thickness and composed of hyaline or light brown, elongated or polyhedral cells. The basal part is hyaline and pseudoparenchymatous. The ostiole is 8—15 μ in diameter and is formed schizogonously. (fig. 17).

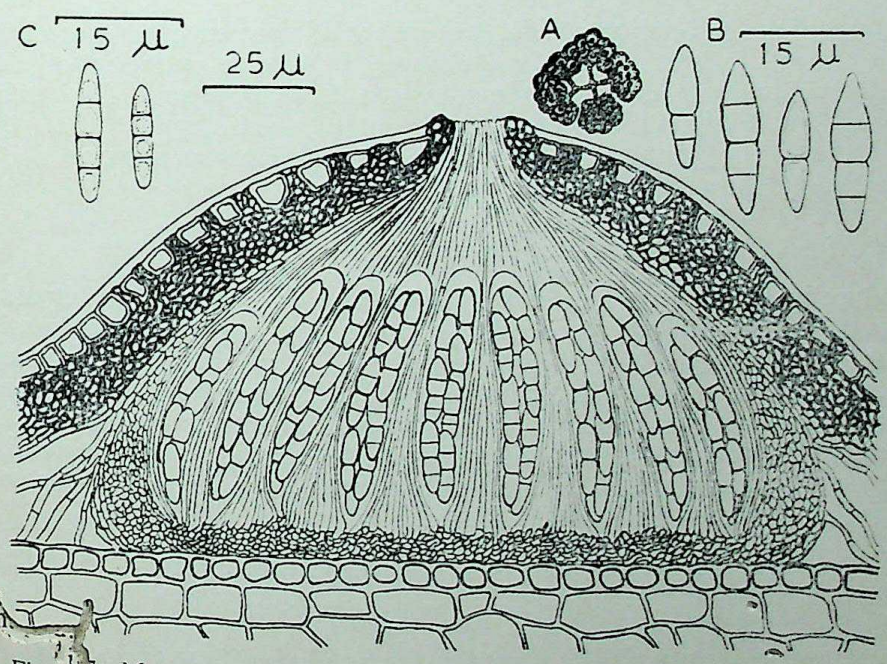


Fig. 17. *Massarina parasitica.* Section through an ascocarp, 500x. Above : A—Ascocarps surrounded by the thallus of *Cephaleuros parasiticus*, 5x. B—Ascospores. 1,000x. C—Pycnidiospores, 1,000x.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The asci are 8—spored, shortly stipitate, cylindrical-clavate, with a double membrane which is thickned and rounded at the apex and measure 40—65 × 8—11 μ. The ascospores are biseriate, straight, fusiform, at first septate in the middle, ultimately becoming 3—septate and 12—18 × 2.5—4.5 μ in size. The paraphysoids are thin, hyaline, filiform and approximately 1 μ in diameter.

The imperfect state of the fungus has been found to occur scattered on the upper surface of the leaves and also in the spots infected with the alga ; in the latter case, it is surrounded by the algal thallus. The pycnidia are globose or subglobose, and 40—70 μ in diameter. The wall of the pycnidium is 6—10 μ in thickness and is composed of brown polyhedral cells. The conidiophores are short and hyaline. The pycnidiospores are hyaline, straight, cylindrical-fusiform, at first septate and unconstricted in the middle, ultimately becoming 3—septate and slightly constricted at the septa. The pycnidiospores measure 11—16 × 2—3 μ. The imperfect stage belongs to the form genus *Stagonospora*.

The fungus grows on malt-extract-agar, forming a greyish colony, but does not sporulate.

The ascomycete belongs to the genus *Massarina*, but differs from the species known so far. The ascospores are similar in structure to those of *M. microcarpa* (Fuckel) Sacc., but are smaller and devoid of the mucous sheath. However, the greatest difference is seen in the structure of the ascocarp, wherein the dark tissue lining the pore is lacking (Bose 1961).

According to Keissler (1930), a number of species of *Metasphaeria* are also known to be parasitic on lichens. Quite a number of these should, eventually, be placed in the genus *Massarina*. The others may also find place in diverse genera as the validity of the genus *Metasphaeria* is open to question (Bose 1961). However, none of the species is identical with *M. parasitica*.

18. *Phillipsiella appendiculata* nov. spec.

Mycelium hypophyllum, superficiale, hyphis ramosis, subhyalinis, 2 μ diam. compositum. Ascomata superficialia, in subiculo sessilia, dispersa, discoidea, 170—400 μ diam., 60—90 μ altitudine. Integumentum fractum cellulis dissolutis hymenii instratum. Paries laterae cellulis elongatis compositus, setis brevis, capitatis ornatus. Asci paralleli, cylindraceo-clavati, bitunicati, 8—spori, paraphysoidibus, filiformibus circumdati. Sporae oblongae, circa medium septatae, hyalinae, 12—15 × 3—5.5 μ.

Hab. in foliis vivis *Quercus dilatatae* Lindl., India, Kumaon, Gwaldam, Dist. Chamoli, April 4, 1964, leg. A. J. Roy.

The fungus grows on the lower surface of living leaves of *Quercus dilatata*. The mycelium is diffused and composed of subhyaline hyphae, measuring approximately 2 μ in diameter, which anastomose at right angles or wide angles. The brownish, flat thyrothecla are scattered and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

measure 170 –400 μ in diameter and 60—90 μ in height. The top of the thyrothecia is composed of a partly torn and partly dissolving roof of brownish cells. At the periphery, the thyrothecium is composed of several layers of intertwined subhyaline hyphae enclosed by an outer layer of elongated brown cells which give rise to short, capitate, unbranched appendages, growing upto 60 μ in length and 3—5 μ in diameter. The basal part is composed of a layer of brown cells, measuring 4—12 μ in diameter. Long, septate, brown hyphae grow downwards from these cells and radiate on the substratum, forming a subiculum, extending 25—50 μ in height. Only a small circular area in the central region of the basal layer does not contribute to the subiculum (fig. 18 A and B).

The asci and thickly parallel, cylindrical club-shaped, truncate at the base, thick-walled and rounded at the apex and measure 30—45 × 10—13 μ. The ascospores are crowded, eight in number, hyaline, oblong-ellipsoid, and septate in the middle and 12—15 × 2.5—5 μ in size. The upper cell is wider than the lower cell. The paraphysoids are present in early stages. These are hyaline, filiform, branched, and sometimes slightly capitate at the apex and measure 2—3 μ in diameter. (fig. 18 C and 19A).

Recently, Müller and Bose (1963) have recorded the occurrence of *P. smilacis* in the central Himalayas, on the leaves of *Smilax aspera* L. The fungus described above, differs from this and other known species of *Phillipsiella* due to the characteristic capitate appendages on the thyrothecia.

19. *Johansonia micheliae* nov. spec.

Mycelium superficiale, hypophyllum, obscurum, hyphis ramosis, subhyalinis vel hyalinis, 2—3 μ crassitudine compositum et setis rectis, fuscis, arcuatis, ad 170 μ longitudine et 4—6.5 μ crassitudine ornatum. Ascomata dispersa, supra subiculum sessilia, discoidea, 250—300 μ diam., 44—60 μ altitudine. Excipulum 10—20 μ crassitudine, hyphis hyalinis vel fuscis compositum. Integumentum 6—10 μ crassitudine, hyphis hyalinis vel fuscis, plusminusve dissolutis constatum. Asci numerosi, paralleli, bitunicati, obovati, 25—33 × 13—20 μ, 8—spori, paraphysoidibus fibbrosis circumdati. Sporae oblongae, circa medium septatae et constrictae, hyalinae, 10—13 × 4—4.5 μ.

Hab. in foliis vivis *Micheliae kisopae* Ham. (*Lauraceae*), India, Kumaon, Chaubattia (Govt. Gardens), April 4, 1964, leg. A. J. Roy.

The fungus grows on the lower surface of the living leaves of *Michelia kisopa* Ham., and forms a superficial, lightly diffused mycelium, composed of hyaline or subhyaline hyphae, measuring 2—3 μ in diameter. The network of hyphae forms a several layered subiculum, extending [illegible]—20 μ in height. On this a disk-like thyrothecium, measuring 25[illegible]—300 × 45—60 μ, is formed. The thyrothecia are surrounded by a few dark [illegible] coloured, pointed bristles, upto 170 μ in length and 4—6.5 μ in diameter [illegible]. At the base the bristles are often expanded into a small disk, upto 14[illegible] μ in diameter. The basal subhymenial layer of the thyrothecium is 4—8[illegible] μ in thickness and is composed of thickly woven, light brown or

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

hyaline hyphae. At the periphery, the thyrothecium is bounded by a curved wall composed of thin-walled, light brown or brown cells, measuring 2—4 μ in diameter. At the top the thyrothecium is covered by a partly dissolving roof which is 6—10 μ in thickness and composed of light brown, plate-like cells, measuring 2—5 μ in diameter.

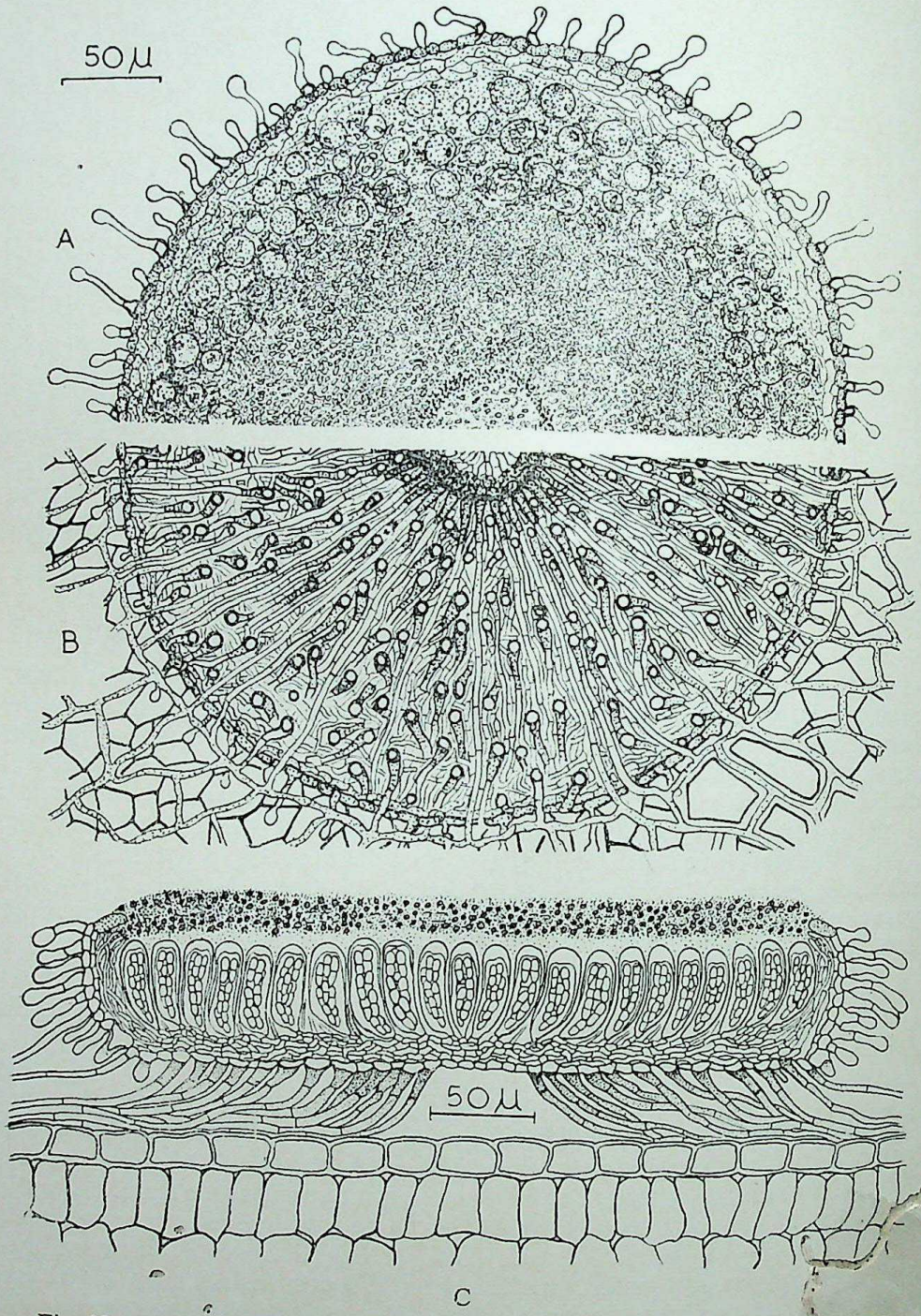


Fig. 18. A, B and C. *Phillipsiella appendiculata*. A—A portion of the thyrothecium viewed from above, 250x. B—A portion of the basal layer of the thyrothecium 250x. C—Section through a thyrothecium, 250x.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The asci are thickly parallel, egg-or club-shaped, variable in length, 25—33 × 13—20 μ in size, and have a short stipe. The asci are bitunicate, distinctly thickened at the apex and contain eight, hyaline, oblong-ellipsoid ascospores measuring 10—13 × 4—4.5 μ in size. The ascospores are septate in the middle and the upper cell is slightly wider. The paraphysoids are attached at both ends and become compressed and gelatinous due to the pressure of tightly packed asci (fig. 19 B).

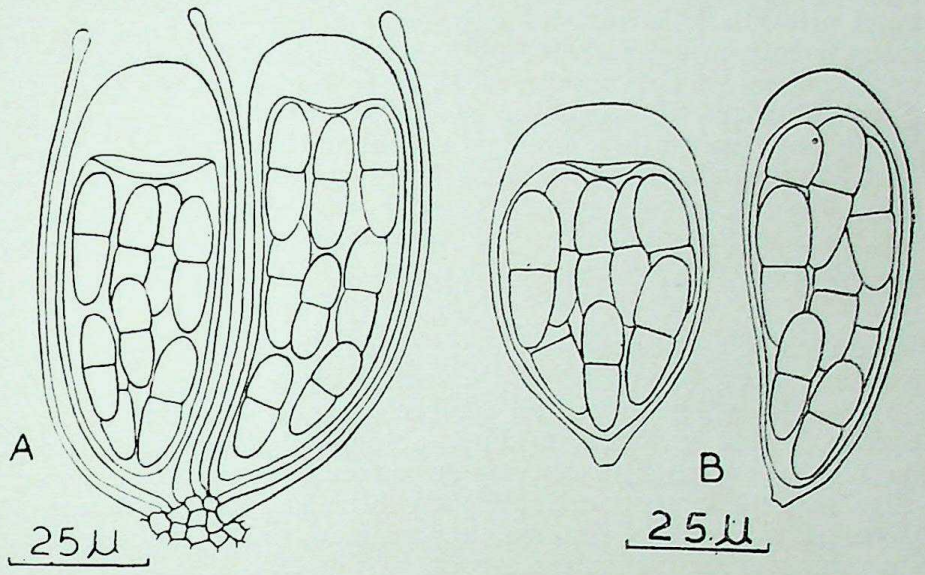


Fig. 19. A—*Phillipsiella appendiculata*. Asci, 750x. B—*Johansonia micheliae*. Asci, 750x.

In general structure of the thyrothecium, the fungus resembles *J, arxi* Bose et Müller (1964), but the size of the ascospores is significantly different. *J. consociata* (Syd.) v. Arx (ap. Müller et v. Arx 1962), has identical ascospores, but differs in having smaller thyrothecia with very short bristles. The present fungus is evidently different from the hitherto recorded species of *Johansonia*.

20. *Microcallis smilacis* nov. spec.

Mycelium superficiale, hypophyllum ex hyphis fusco-luteis, 2—4 μ crassitudine compositum. Perithecia dispersa, subglobosa, 70—140 μ diam. et 40—95 μ altudine, sub mycelio nascentia et poro rotundato aperta. Paries peritheciorum cellulis subfuscis compositus, apicaliter tenuis, lateraliter vel basaliter incrassatus, setulis fuscis, septatis, verrucosis, obtusis vel fastigiatis et rare curvatis, 25—80 × 3—6 μ ornatus. Asci cylindracei vel obclavati, bitunicati, 50—75 × 9—14 μ, octospori. Sporae oblongae, circa medium septatae, 14—20 × 2—4 μ, cellulis ultimis leniter incrassatis. Paraphysoides fibbrosae, perithecia matura plurimitosa.

Hab. in foliis viviis *Smilacis asperae* L., India, Kumaon, Chaubattia (Ranikhet), September 18, 1963, leg. S. K. Bose.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The mycelium of the fungus forms a diffused layer on the lower surface of the living leaves of *Smilax aspera* L., and is composed of brownish-yellow, setose hyphae 2—4 μ in diameter, which frequently anastomose at approximately right angles, forming a thin net-work. The ascocarps are scattered, and are hemispherically shield-shaped and measure 70—140 × 50—95 μ. The wall is thin at the apex, gradually becoming thicker at the base. The outer portion of the wall is composed of thick-walled, dark brown, polyhedral cells upto 8 μ in diameter. These are beset with dark brown, unbranched, septate, blunt and curved appendages, measuring 25-80 × 3-6 μ. The surface of the appendages is encrusted or warty in appearance. The inner portion of the ascocarp is nearly spherical and is composed of a layer of light brown, thin-walled, polyhedral cells, measuring 4-8 μ in diameter. This inner wall forms a distinct layer at the base and the sides. The space between the outer and inner layers of the ascocarp wall is full of a net-work of meandering, yellowish-brown, thin-walled hyphae, approximately 2-4 μ in diameter. The ostiole is apical and 10--15 μ in diameter.

The asci are cylindrical, obclavate, gradually tapering and rounded at the apex, and are subsessile at the base. eight spored and measure 50--75 × 9—14 μ. The ascus wall is bitunicate and thickened at the apex. The ascospores are crowded, hyaline, oblong-ellipsoid, smooth, septate in the middle, and slightly tapering or rounded at the ends and measure 14—20 × 2-4 μ. The upper cell is slightly broader than the lower. The paraphysoides are hyaline, filiform, approximately 2 μ in diameter and become evanescent in the mature ascocarp (Fig. 20).

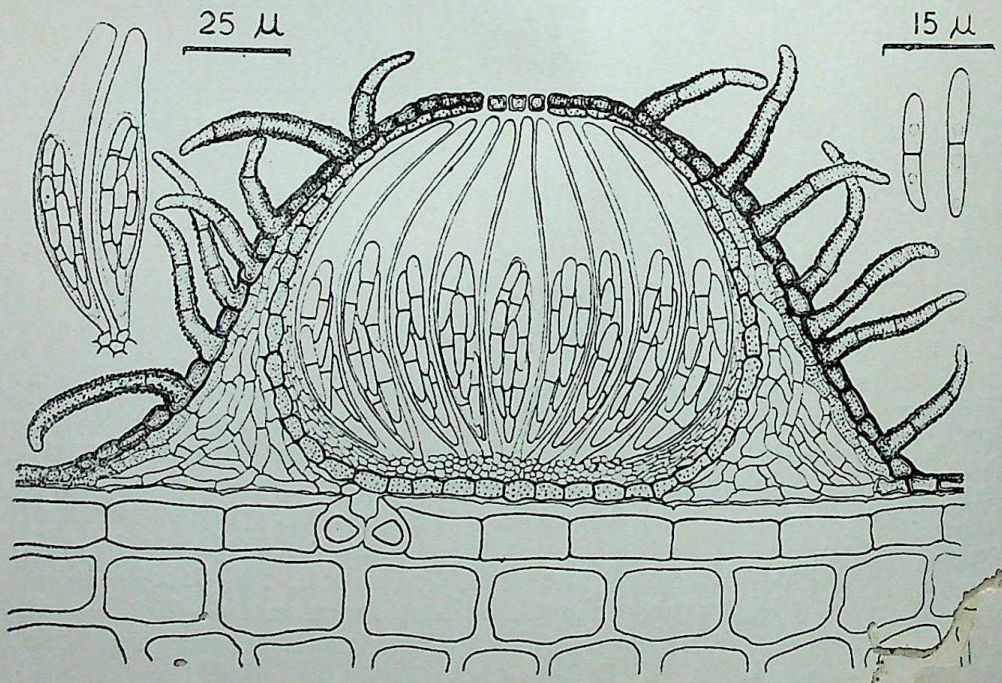


Fig. 20. *Microcallis smilacis*. Section through an ascocarp 500x. Upper left—Asci, 500x. Upper right—Ascospores, 1,000x.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

So far only one species of *Microcallis* is known from India, viz., *M. negii* (Müller et Bose) Müller et v. Arx (1962) [= *Chaetothyrina negii* Müller et Bose (1959)]. The species on *Smilax aspera* differs from all species of *Microcallis*, so far recorded.

A fungus similar to the above has been found to occur on the lower surface of living leaves of *Quercus dilatata* Lindl., (Naini Tal, 11.3.1964, leg. B. S. Khati). The ascocarps are lighter in colour. The appendages are also lighter in colour and smooth in early stages, becoming encrusted, warty and darker in mature ascocarps. Except for these minor differences, the fungus agrees in all other aspects with the species on *Smilax* and is considered to be identical.

According to Hansford's description and drawing (1946), *M. scutiae* appears to be a nearly related species. *M. scutiae* has been made the type of the genus *Recifea* by Batista and Ciferri (1962). In the key the authors say, "Mycelium setose, perithecia setose," and they give the following remarks : "We have not studied the single species ascribed to this genus, so that the interpretation of the short and stout appendage like warts on the perithecium, indicated by Hansford are of doubtful judgement. They are described as continuous or uniseptate, so that we suppose these warts are, really, young or abortive perithecial setae." These assertions are, of course, unwarranted and *Recifea* Bat. and Ciferri is only a synonym of *Microcallis* Syd. *Microcallis scutiae* has strongly convex ascocarps. Sydow (1926) has, in fact, noted the tendency of some species of this genus to form strongly convex shield-like ascocarps. *M. smilacis* forms a close link between *M. scutiae* and other known species of the genus.

21. *Otthia indica* nov. spec.

Perithecia solitaria vel aggregata, conica stromatibus, basaliter sessilis, 400-650 μ altitudine, 400-500 μ diam., ostiolo elongato-conico, 85-120 μ crassitudine. Paries peritheciorum 40-70 μ crassitudine, atro-brunneus, cellulis elongatis compositus. Asci bitunicati, cylindracei, 90-130 × 11.5-17.5 μ, Sporae ellipsoideae, circa medium septatae, primo hyalini, demum sub-fuscae, 22-33 × 7.5-12.5 μ. Paraphysoides hyalinae, fibrosae, 2 μ diam.

Hab. in ramis vivis *Quercus glauci* Thunb. subsp. *annulatae* Sm., et *Loranthi vestiti* Wall. in Quercu parasiticans. India, Kumaon, Ranikhet, December 12, 1962, leg. S. K. Bose.

The fungus occurs in the cortex of living branches of *Q. glauca* Thunb., parasitised by *Loranthus vestitus* Wall. and also in the cortex of the latter. The ascocarps are formed singly or in small groups. These are pear-shaped, flattened and embedded at the base and measure 400—650 × 400—500 μ in size. The wall of the ascocarp is 40—70 μ in thickness and is composed of thick-walled, dark brown, elongated or polyhedral cells, measuring 4—8 × 2—4 μ. The inner layers and the basal layers are composed of light brown or hyaline, thin-walled cells. Towards the apex

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the perithecia project into a conical or elongated neck which is upto 150 μ in diameter. The ostiole is 85--120 μ in width and is lined with filiform, septate, hyaline periphysoids, approximately 2 μ in diameter.

The asci are cylindrical with a double membrane which is slightly thickened at the apex and measure 88—129 × 11.5—17.5 μ. The ascospores are biseriate in early stages, becoming over-lappingly uniseriate later and are septate in the middle or slightly below the middle. The ascospores are distinctly constricted at the septa, ellipsoid-biconic or spindle-shaped, tapering or broadly rounded at the ends. These are hyaline and 4-guttulate in the beginning, becoming yellowish or yellowish-brown and 2-guttulate later on. Ultimately, the mature ascospores become unguttulate and brown and 22—33 × 7.5—12.5 μ in size. The paraphysoides are approximately 2 μ in diameter (fig. 21).

It is not easy to find a good genus for this fungus. Although in typical cases, a distinctly crowded growth of the fruit bodies is lacking, a distinct basal stroma is recognisable and the ascocarps often build loose groups, whereby we are placing this fungus in the genus *Otthia* Fuckel. The species of this genus also possess relatively big ascospores that remain hyaline for a long time, later becoming light and ultimately dark

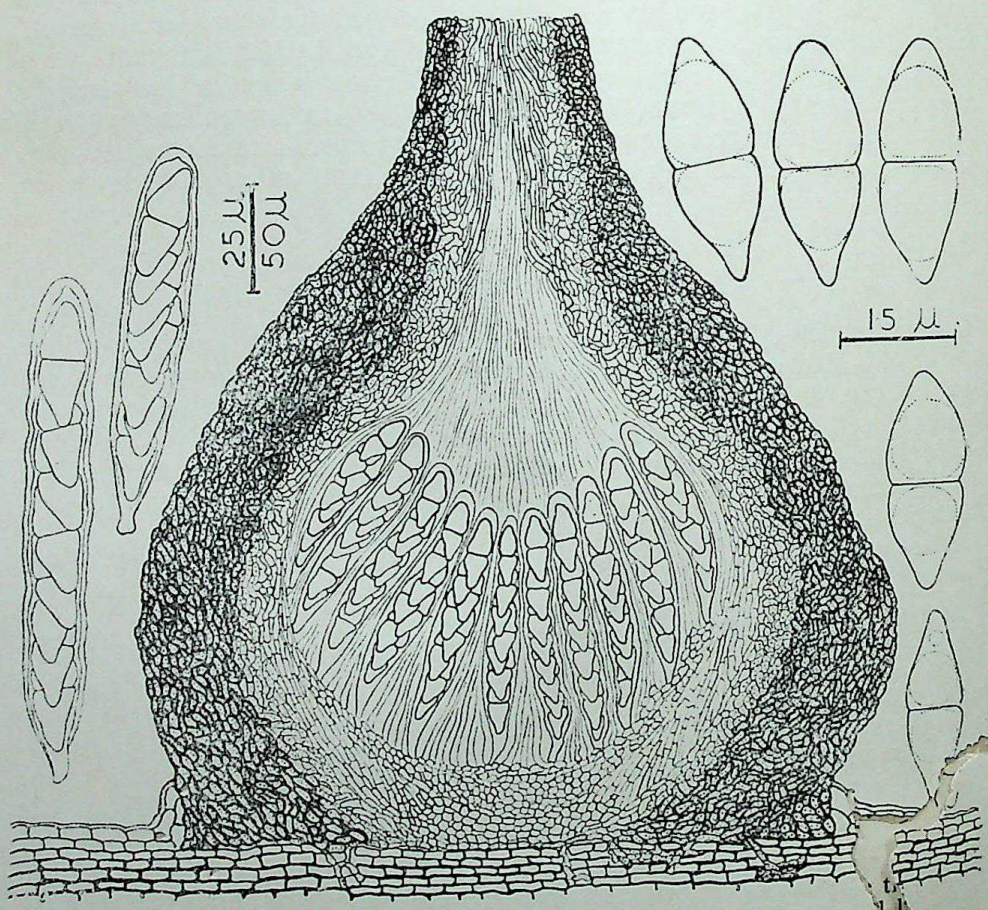


Fig. 21. *Otthia indica*. Section through an ascocarp, 250x. Left—Asci, 500x. Right—Ascospores, 1,000x.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

brown in colour. The fungus described above is different from the other species of *Otthia*. We have also compared it with *Caryospora striata* (Niessl) Scheinpflug ; in the original material of this (=*Amphisphaeria striata* Niessl), the spores are longer, more slender and often exhibit pseudosepta at the ends (whereby also it belongs to *Caryospora* de Not.). *Amphisphaeria striata* exhibits even less of an ascostroma. On the contrary, the collections from Pakistan, ascribed to this species by Scheinpflug (1958), actually belong to *Otthia indica*.

22. *Schizothyrium pomi* (Mont. ex Fr.) v. Arx-Proc. Kon. Ned. Ak. Wet. C *62* : 336 (1959).

Material examined : Living leaves of *Michelia kisopa* Ham. (*Lauraceae*), collected in Chaubattia (Government Gardens), April 5, 1964, leg. A. J. Roy.

The thyrothecia of *S. pomi* occur along with those of *Johansonia micheliae* Bose et Müller and it is usually not easy to distinguish the two fungi macroscopically.

The very indistinct mycelium ramifies over the whole of the lower surface of the leaf and also penetrates within the cuticle. The shield-shaped, round or usually elliptical thyrothecia are 100-400 μ in diameter and attain a height of 40-50 μ. Their very dark upper wall is composed of one or two layers of dark brown cells, measuring 4-8 μ in size. Towards the periphery, the upper wall ramifies out into a very thin film, the cells of which are often in rows, crossing each other. From the thin basal layer, club-shaped asci arise parallel to one another. The asci are 20-35$\times$11-18 μ in size. The ascospores are eight in number, bicelled, hyaline and measure 11-14$\times$3-4 μ.

Recently, Bose (1962) has recorded the occurrence of *S. crenulatae* in the central Himalayas. According to von Arx (1959), *S. pomi* has a wide geographical range and is a polyphagus species, occurring on numerous hosts, apparently living on their exudates. This fungus, which has been well illustrated by von Arx (1959) and also by Müller and von Arx (1962), has so far not been recorded from India, but its range would surely be found to be wider. We have already found this fungus on two species of the genus *Myrsine* (*Myrsinaceae*) in the central Himalaya, viz., *M. africana* L. (Chaubattia, Drug Farm, March 30, 1963, leg. S. K. Bose), and *M. semiserrata* Wall. (Government Gardens, Chaubattia, and Jhuladevi Bridle Path, Ranikhet, March 30, 1963 and March 25, 1963, leg. S. K. Bose).

23. *Schizothyrium perexiguum* (Rob.) v. Hohn.—Ann. Mycol. *15* : 297 (1917).

Material examined : Leaves of *Myrsine semiserrata* Wall., Jhuladevi Bridle Path, Ranikhet, March 3, 1963, leg. S. K. Bose.

The thyrothecia occur on the lower surface of living leaves, scattered on the cuticle and are flat, shield-shaped, round in outline, measuring 250-400$\times$30-50 μ. The upper wall, which is composed of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

brown polygonal cells of 4-8 μ width, breaks up by the formation of a circular rupture at the top, giving rise to a number of long, irregularly radial fissures. Subsidiary fissures arise from the radial fissures, resulting in the fragmentation of the thyrothecial covering into flake-like pieces. Towards the edges, the thyrothecial wall ramifies into meandering, brownish or subhyaline, irregularly swollen hyphae. These anastomose into a thin net-work which is nearly hyaline at the periphery (Fig. 22).

The asci are 8-spored, parallel, egg-shaped or nearly club-shaped with a double wall which is thickened at the apex and measure 18-33 × 9-15 μ. The ascospores are hyaline, oblong-ellipsoid, septate approximately in the middle, and measure 9-13 × 4-5 μ. The upper cell is slightly broader than the lower. The septate paraphysoids gelatinise on maturity.

This fungus has a growth akin to *S. pomi*, but differs, above all, in having lighter coloured thyrothecia as well as the meandering composition of its upper wall. In addition, the ascospores are smaller. *S. perexiguum* has been described in detail by v. Arx (1959) and also by Müller and v. Arx (1962). It seems that the present record of this fungus from India is the first from Asia also.

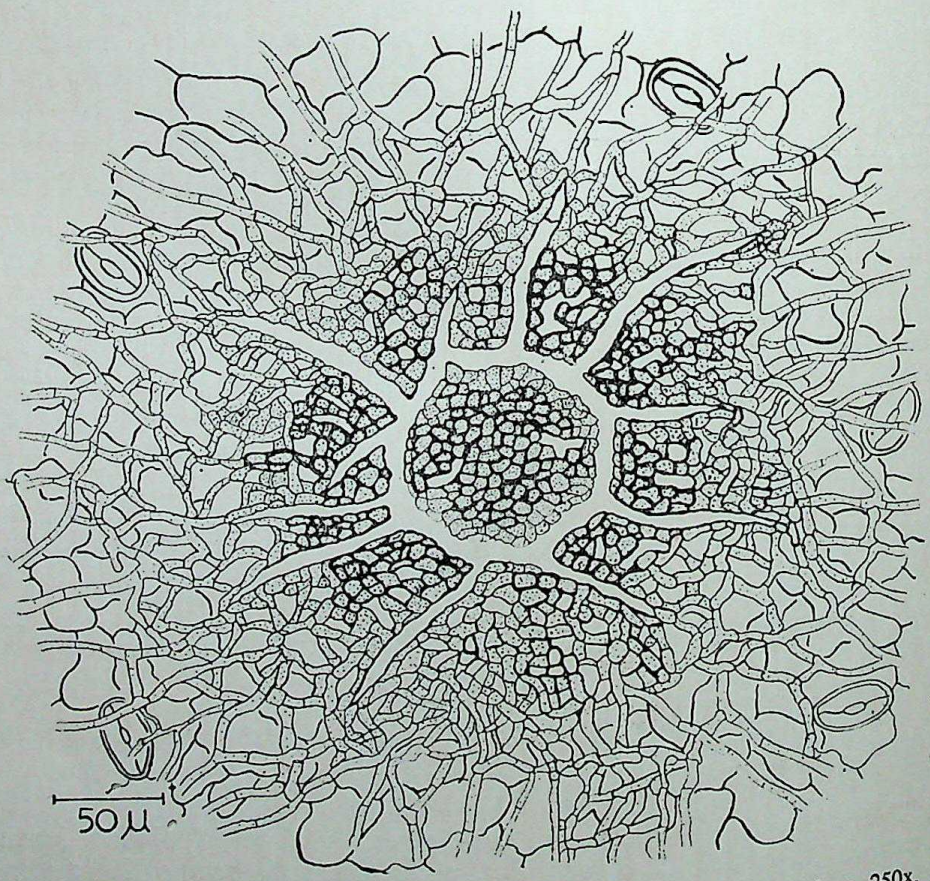


Fig. 22. *Schizothyrium perexiguum*. Surface view of a thyrothecium, 250x.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

24. *Chaetothyrina musarum* (Speg.) Theiss.—Ann. Mycol. *11* : 495 (1913).

Material examined : Leaves of *Smilax aspera* L., Chaubattia (Ranikhet), April 4, 1964, leg. S. K. Bose.

As mentioned earlier in this paper, this fungus occurs on the living leaves of *Smilax aspera* L., along with the fungus *Phillipsiella smilacis*. *Chaetothyrina musarum* has not been recorded on *S. aspera* so far, although Müller and Bose (1963), have reported this fungus on *S. parvifolia* Wall. Like *Schizothyrium pomi*, this fungus also appears to have a wide host range. We have already found this fungus on *Quercus dilatata* Lindl., (Gwaldam, East Garhwal, April 30, 1964, leg. A. J. Roy) ; *Euonymus tingens* Wall., (Chaubattia, near Government Gardens, Nov. 28, 1962, leg. S. K. Bose) ; *Myrsine africana* L. [(Chaubattia, Drug Farm, March 30, 1963, leg. S. K. Bose (besides *Schizothyrium pomi*)] ; and *Myrsine semiserrata* Wall., (Chaubattia, Drug Farm, April 5, 1964 and Chaubattia, Government Gardens, April 6, 1964, leg. S. K. Bose). The fungus on these collections differs slightly in having longer or shorter setae and in small variations in the size of the ascospores but these differences are not sufficient to indicate species different from *Chaetothyrina musarum*.

25. *Ellisiodothis smilacis* (de Not.) v. Arx et Müller—Beitr. Krypt. Fl. Schweiz, *11*, 96 (1954).

Material examined : Dead branches of *Smilax aspera* L., Chaubattia (Ranikhet), October 10, 1963, leg. S. K. Bose.

A very brief reference has been made about the occurrence of this fungus on two species of *Smilax* in central Himalayas by Müller and Bose (1963). At the first glance the fungus in the present collection appears to be different from *Ellisiodothis smilacis* described by Saccardo (1883), Müller and v. Arx (1954) and Müller and Bose (1963), inasmuch as the ascospores described by these authors are much smaller. However, it appears that the ascospores and asci of *E. smilacis* take some time to mature and it all depends whether one is examining the fungus at an earlier or a latter stage. The measurements given by Luttrell (1944) for this fungus [=*Myiocopron smilacis* (de Not.) Sacc.], on *Smilax rotundifolia* are identical with those of the fungus on *S. aspera*. It has been, therefore, deemed advisable to give an adequately detailed description of this fungus :

The ascocarps are superficial, lens-shaped, 400-500 × 90-112 μ. The ascocarp wall is 20-35 μ thick and composed of radial, brown, elongated-polyhedral cells. The ostiole is apical and 30-40 μ in diameter. The base of the ascocarp is composed of several layers of hyaline hyphae ; the lower most layer is brownish and at places produces brownish hyphal knots which penetrate the epidermal and subepidermal cells. The asci are cylindrical or ellipsoid, broadly rounded at the apex and measure 55-88 × 22-27 μ. The ascospores are eight in number, biseriate or crowded, hyaline, ellipsoid or oval, rounded at the ends,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sometimes broader at the apex or slightly constricted in the middle, and 15-24 × 8.5-11 μ in size. The paraphysoids are numerous, hyaline, filiform and 2-3 μ in diameter (Fig. 23).

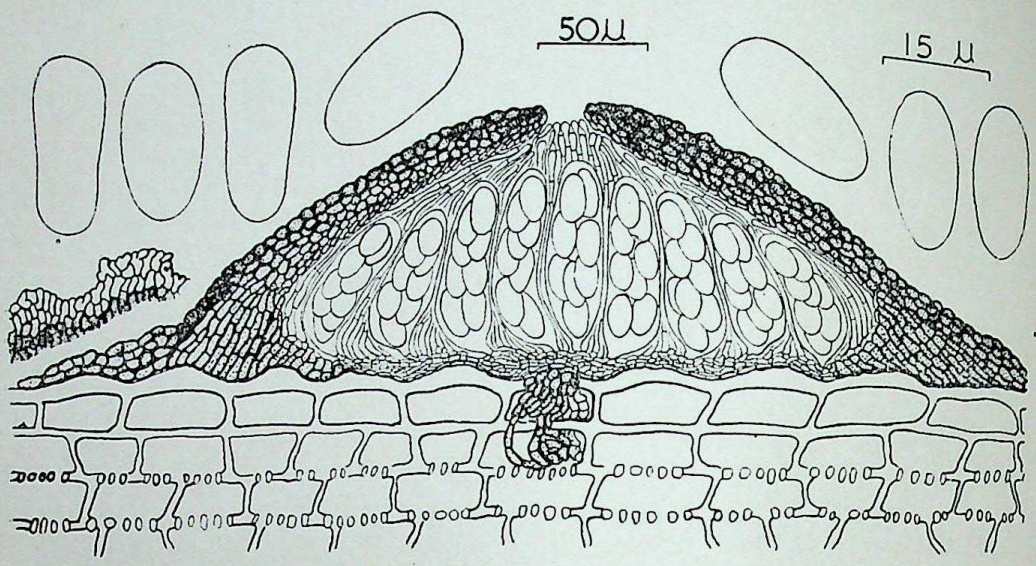


Fig. 23. *Ellisiodothis smilacis*. Section through an ascocarp, 250x. Above—ascospores, 1,000x. Left middle—Periphery of ascocarp, 250x.

Summary

In this paper a number of ascomycetes have been discussed, viz., *Lophodermium querci* sp. nov., *Acantharia quercus-dilatatae* sp. nov., *Massarina parasitica* sp. nov., *Phillipsiella appendiculata* sp. nov., *Johansonia micheliae* sp. nov., *Microcallis smilacis* sp. nov., *Otthia indica* sp. nov., *Schizothyrium pomi* (Mont. ex Fr.) v. Arx., *Schizothyrium perexiguum* (Rob.) v. Höhn., *Chaetothyrina musarum* (Speg.) Theiss., and *Ellisiodothis smilacis* (de Not.) v. Arx.

Hill Fruit Research Station, Chaubattia,
U. P., and Institute of Special Botany,
Swiss Federal Institute of Technology,
Zürich, Switzerland.

References

v. Arx, J. A. (1954) Revision einige Gattungen der Ascomyceten. *Acta Bot. Neerl.* **3** : 83-93.

——— (1959) Ein Beitrag zur Kenntnis der Fliegenfleckenpilze. *Proc. Konigl. Ned. Ak. Wet.* Amsterdam, ser. C. **62** : 333-340.

——— and E. Müller (1954) Die gattungen der amerosporen Pyrenomyceten. *Beitr. Krypt. Fl. Schweiz.* **2** : 1-434.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BATISTA, A. C. AND R. CIFERRI (1962) The *Chaetothyriales. Beih. zur Sydowia* (*Ann. Mycol.* ser. II) *III* : 1-129.

BOSE, S. K. (1961) Studies on *Massarina* Sacc. and related genera. *Phytopath* Z. **41** : 151-213.

——— (1962) Fungi on *Crataegus crenulata* Roxb. in Himalaya. *Indian Phytopath.* **15** : 141-155.

——— AND E. MÜLLER (1964) Central Himalayan Fungi—I. *Indian Phytopath.* **17** : 3-22.

HANSFORD, C. G. (1946) The foliicolous ascomycetes, their parasites and associated fungi. *Imp. Myc. Inst. Mycol. paper* no. 15.

KEISSLER, L. (1930) Die Flechtenparasiten. *Rabenhorsts Kryptogamenflora* VIII Band, 1-712.

LUTTERELL, E. S. (1944) The morphology of *Myiocopron smilacis* (de Not.) Sacc. (Die-back of *Smilax rotundifolia*). *Amer. J. Bot.* **31** : 640-649.

MÜLLER, E. (1958) Pilze aus dem Himalaya II. *Sydowia*, **12** : 160-184.

——— AND J. A. VON ARX (1962) Die gattungen der didymosporen Pyrenomyceten. *Beitr. Krypt. Fl. Schweiz.* **11** (2) : 1-922.

——— AND S. K. BOSE (1959) Ueber eine Erkrankung an *Taxus* im Himalaya. *Indian Phytopath.* **12** : 13-18.

——— AND S. K. BOSE (1963) Ueber einige Ascomyceten auf *Smilax* Arten aus dem Himalaya. *Indian Phytopath.* **14** : 245-250.

SACCARDO, P. A. (1883) *Sylloge Fungorum* **2**, Patavii.

SCHEINPFLUG, H. (1958) Untersuchungen über die Gattung *Didymosphaeria* Fuck. und einige verwandte Gattungen. *Ber. Schweiz. Bot. Ges.* **68** : 325-385.

SYDOW, H. (1926) Fungi in itinere costaricensi collecti. *Ann. Mycol. Berl.* **24** : 283-426.

TEHON, L. R. (1935) Monographic rearrangement of *Lophodermium. Ill. Biol Monogr.* **8** (4) : 1-151.

TERRIER, CH. (1942) Essais sur la systematique des *Phacidiaceae* (Fr.) sensu Nannfeldt. *Beitr. Krypt. Fl. Schweiz.* **9** (2) : 1-99.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON PUCCINIA HORDEI II—FORMATION AND GERMINATION OF TELEUTOSPORES*

L. M. JOSHI

(Accepted for publication November 10, 1965)

INTRODUCTION : In the life cycle of the rust fungi, teleutospores usually play a significant part in the perpetuation of the disease from one season to the next and also in bringing about variability within the fungus. In some rusts, the teleutospores remain dormant and difficulty is experienced in inducing them to germinate. Workers such as Beck (1924), Mains ond Jackson (1924), Documet and Foex (1925), Tranzschel (1934), D'Oliveira (1949), Critopoulus (1956) and Dickson (1956) have succeeded in infecting species of *Ornithogalum* or *Dipcadi*. However, Waterhouse (1929) in Australia failed to germinate the teleutospores. In India, Joshi *et al.* (1959) also did not succeed in germinating teleutospores of the rust. The present studies were taken up to secure information on conditions favourable for their formation and subsequent germination.

MATERIAL AND METHODS : The method recommended by Mehta (1940) for wheat rusts was followed for the study of germination of teleutospores of *P. hordei*. They were raised on varieties Hyb. 5 and J. R. 31 under natural conditions.

EXPERIMENTAL : Under Delhi conditions the teleutosori of leaf rust of barley begin to appear after the 3rd week of March. Ali varieties of barley, however, do not induce production of telia. Field observations on many varieties have indicated that only two of them namely, J. R. 31 and Hyb. 5 were found suitable for their development. The telia appear in the lower leaves or culms of the plants, as compact pin-head like structures which become concentric. Under Delhi conditions they appear ordinarily on drying parts of the plant, but when plants are made to grow at low temperatures, they appear even in green parts.

At St. Paul, Minnesota, a field experiment was conducted in which twenty-three varieties (or species) of barley, namely, Trail, Trophy, Parkland, Egypt-4, Oderbrucker, Erie, Black Hull, Cebeda Capa, Larker, Anoidin, *Hordeum agrocrithium*, Kinred, Olebrum, II-51-4 B, C. I. 4979, C. I. 4974, C. I. 4797, C. I. 9792, C. I. 4781, 4976, JR 31, Hyb. 5 and N D. B. 112) were sown in two lots on May 9 and May 16, 1963 and in one lot at Rosemount Farm on May 13, 1963. They were sown in 9 feet rows, 1 foot apart and were inoculated at 4-leaf stage. None of these varieties produced telia either at

*Part of the thesis submitted in partial fulfilment of the requirement for Ph. D. degree of Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Rosemount or St. Paul Farms and the crop was harvested in the last week of August. The two Indian varieties (J R. 31 and Hyb. 5) were severely affected by mildew.

Another lot of the same varieties was again sown in Minnesota on August 20, 1963 and inoculated after about a month in 4-leaf stage. In this plot on four varieties, viz., Trophy, Trail, Parkland and Erie appearance of telia was observed in the middle of October. When the last observations were taken on 12th November, 1963 varieties Trophy, Trail, Parkland, Erie produced telia in abundance while JR. 31 and Hyb. 5, ND. B. 112 came next. Other varieties either did not produce telia or did so only in traces. In case of Trophy the telia appeared on the green parts of the plant.

Viability of teleutospores : 1958-59 Crop : Teleutospores collected from the field in Delhi (Indian Agricultural Research Institute) on April 3rd and 15th were brought to the laboratory and kept for germination at ranges of temperature varying from 5 to 25°C. Part of the material was sent to Simla (7,000 ft. A. S. L.) and stored in refrigerator at 5°C.

The material stored at Delhi was kept in moist chambers for germination at temperatures of 5, 10, 15, 20 and 25°C.

The material was alternately frozen and thawed as recommended by Mehta (1940). Results of the tests carried out during 1959-60 with the material collected from 1958-59 Crop in April 1959 are summarised below :—

Material collected on April 3, 1959

1.	Germination tested at 5°, 10∓1°, 15°C and 25∓1°C in hanging drop method in (distilled water) on April 16, 1959. Observations taken for nearly ten days.	No germination.
2. (a)	Germination tested on October 3, 1959 at 10∓1°, 15°C and 25°∓1°C.	No germination.
(b)	Material given two freezings and two dryings before testing for germination.	No germination.
3. (a)	Germination tested on December 10, 1959 at 10°C, 8-20°C (room temperature), 10-20°C (green house) and 25∓1°C.	No germination.
(b)	Material given 3 freezings and 3 dryings (24 hours each) before testing germination at temperature of 10°C, 8-20°C (room temperature), 10—20°C (Greenhouse) and 25∓1°C.	No germination.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

4. (a) Germination tested on January 24, 1960 at 5°C. 10°C, 8-16°C (room temperature) 10-20° (average green house temperature) and 25∓1°C. No germination.

(b) Germination tested on January 24, 1960 at 5°C, 10°C, 8-16°C (room temperature) 10-20°C (average green house temperature) and 20°±1°C. after giving 3 freezing and three drying treatments (each of 24 hours duration). No germination.

Material collected on April 3, 1959 and stored at Simla in a refrigerator was tested for germination first at Simla and then at Delhi. This was tested at temperatures of 5-25°C from September 1959 to February 1960 by giving 2 to 3 days' thawing and then subjected to several alternate wettings and dryings but here also no germination occurred.

1959-60 Crop : Telial material was collected on the 29th March and 7th April 1960 part of which was sent to Simla and the remaining retained at Delhi. The tests were carried out in April and May 1960 and from October 1960 to March 1961 at least once in each month. No success was achieved. Material collected from 1960-61 Crop also failed to show any germination.

As the material collected from the field in the months of March or April did not show any germination it was felt that telia raised at low temperatures might show some germination. The two varieties of barley namely, J. R. 31 and Hyb. 5 were sown on August 30, September 3, and September 10, 1960, in small plots in the Division of Mycology and artificially inoculated in October with a mixture of races H_1 and H_2. Telia were first observed (on the plants sown on August 30) in the last week of December and material was collected on January 14, 1961. It was stored at 0°C. Part of the material was soaked in water and frozen in ice and the other parts was kept dry in sealed tube at 0°C. Viability in both the lots was tested every month from January onwards at temperature ranges of 5-25°C and except in one case, details of which are given in Table given below, negative results were obtained.

TABLE : Germination of teleutospores of *P. hordei* produced at low temperature and stored for about five months after 3 wettings and 3 dryings.

Examined on	Temperature range			Remarks
	15-1°C	18-20°C	25-1°C	
29/5	Nil.	Nil.	Nil.	
30/5	Nil.	One meso-spore.	Nil.	
31/5	Nil.	Tr.	Nil.	
1/6	Nil.	Tr.	Nil.	
2/6	Nil.	Same*	Nil.	

*Tr.=Trace, less than 1 per cent.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The work carried out from 1958 to 1962 with the field material collected in the months of March and April did not show any germination which indicates that they are not viable but the material formed in winter months germinated, though only in traces, at a temperature range of 18-20°C after a dormant period of 4 or 5 months. It has been found that the teleutospores lose their viability when exposed to summer tempeatures.

ACKNOWLEDGEMENT : My thanks are due to Dr. R. Prasada Indian Agricultural Research Institute, Drs. E. C. Stakman and Helen Hart, University of Minnesota, for their valuable suggestions. Visit to Minnesota was made possible under a Rockefeller Foundation Grant for which I record my thanks.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

REFERENCES

BECK, O. (1924) Ein Infektionsversuch mit *Puccinia simplex*. *Ann. Mycol. Berl.* **22** : 291-292.

CRITOPOULUS, P. (1956) Perpetuation of Brown Rust of Barley in Attica. *Mycologia* **48** : 596-600.

DICKSON, J. G. (1956) Diseases of Field Crops. McGraw Hill Book Company Inc. New York.

DUCOMET, V. AND E. FOEX (1925) Introduction a une Etude Agronomique Des Rouilles Des Cereales. *Ann. Des Epiphytics* (*et de Phytogenetique*) **11** : 311-411.

D'OLIVEIRA, B. (1949) Aecidia of Barley Dwarf Rust. *Nature* (*Lond.*) **164** : 880-881.

JOSHI. L. M., D. P. MISRA, K. R. SREEKANTIAH, V. C. LELE AND D. KAK (1959) Studies on *Puccinia hordei* Otth, the leaf rust of barley in India. *Indian Phytopath.* **12** : 69-75.

MAINS, E. B. AND H. S. JACKSON (1924) Aecial stage of leaf rust of rye, *P. dispersa* Eriks. and Henn. and barley *P. anomala* Rostr. in United States. *J. Agric. Res.* **28** : 1119-1126.

MEHTA, K. C. (1940) Further Studies on Cereal Rusts in India. *Sci. Mono.* (I. C. A. R.) 14, 240 pp.

*TRANZSCHEL, W. (1934) Alternate Hosts of Cereal Rusts and their Distribution. *Bull. Pl. Prot. Leningr. Ser.* II. **5** : 4-40.

WATERHOUSE, W. L. (1929) Australian Rust Studies I. *Proc. Linn. Soc. N. S. W.* **54** : 615-680.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE GENUS MACROLEPIOTA IN INDIA

R. N. GHOSH AND N. C. PATHAK

(Accepted for Publication November 5, 1965)

The genus *Macrolepiota* was established by Singer in 1948. Several earlier authors called the Agaricaceae either *Lepiota* or *Agaricus*. If this is accepted, the genus *Lepiota* would be of enormous size containing heterogenous elements. Singer (1951, 1962) does not agree with this and he has, therefore, divided the Agaricaceae into four tribes: (*i*) *Leucocoprineae*, (*ii*) *Agariceae*, (*iii*) *Lepioteae* and (*iv*) *Cystodermateae*. In the tribe *Leucocoprineae*, he has kept six genera namely, *Clarkeinda*, *Chlorophyllum*, *Volvolepiota*, *Macrolepiota*, *Leucoagaricus* and *Leucocoprinus*, in one or other of which, the species described earlier under *Lepiota* are placed. Others have, however, given different views about the position of the genus *Lepiota* from which some species have been put by Singer (1962) in *Macrolepiota*. Kaunrad and Maublanc (1948) consider the upgrading of the sub-genus *Hiatula* and *Leucocoprinus*, by some earlier workers to generic rank rather premature and they have, therefore, retained them provisionally as sub-genus of *Lepiota* keeping *Lepiota excoriata*, *L. procera*, *L. mastoidea* and *L. rachodes* under the section *procerae* of the sub-genus *Leucocoprinus*. Moreau (1954) more or less follows Kaunrad and Maublanc and in his treatise, "Les Champignons" keeps *Leucocoprinus* only as the sub-genus of *Lepiota* consisting of species like *L. procera*, *L. rachodes*, *L. excoriata* along with *L. naucina*. Hongo (1959), however, in his treatment of the "Agaricales of Japan" differs from Singer in putting secondary emphasis on microchemical and microscopical characters of the spores. He divides the family Agaricaceae into two tribes : (*i*) *Lepioteae* with genera having white or rarely greenish or ochraceous coloured spore print and (*ii*) *Agariceae* for those with dark coloured spores. In his tribe *Lepioteae* he keeps *Chlorophyllum*, *Lepiota* (including Singer's *Macrolepiota*, *Leucocoprinus*, *Leucoagaricus* and *Lepiota*) *Melanophyllum*, *Cystoderma* and *Phaeolepiota* and the genus *Agaricus* in the tribe *Agariceae*. Hongo is of the opinion that Singer's differentiation of *Macrolepiota*, *Leucocoprinus*, *Leucoagaricus* and *Lepiota* (chiefly based on the characters of spore, the presence or absence of the clamp connections etc.) are infra generic ones of the *Lepiota sensu lato*.

Singer (1962) differentiates the genera of his sub-tribe *Leucocoprineae* on the basis of spore colour, absence of clamp connections and shape as well as the size of the cap. The tribe *Leucocoprineae* is characterized by the presence of pseudo-amyloid spores with complex wall and a germ pore, deep blue or distinctly metachromatic in cresyl blue stain, spore print usually white, volva not well developed or rudimentary or well developed as in the case of *Volvolepiota* and *Clarkeinda*.

The genus *Macrolepiota* is characterized by a white spore print, spores are metachromatic in cresyl blue with a broad germ pore, smooth, large in size, varying from 8 μ to 22 μ. Cystidia absent on the side of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 1 *Macrolepiota procera*

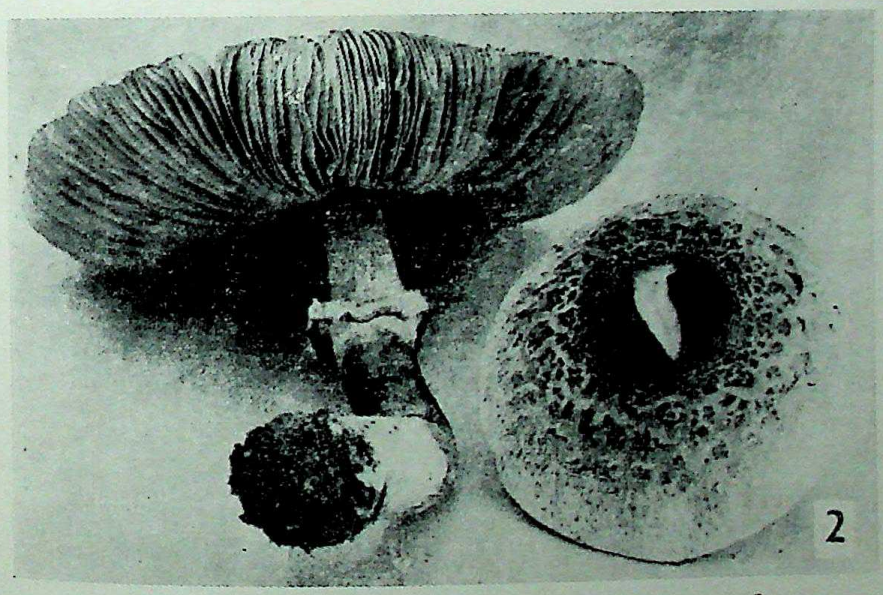
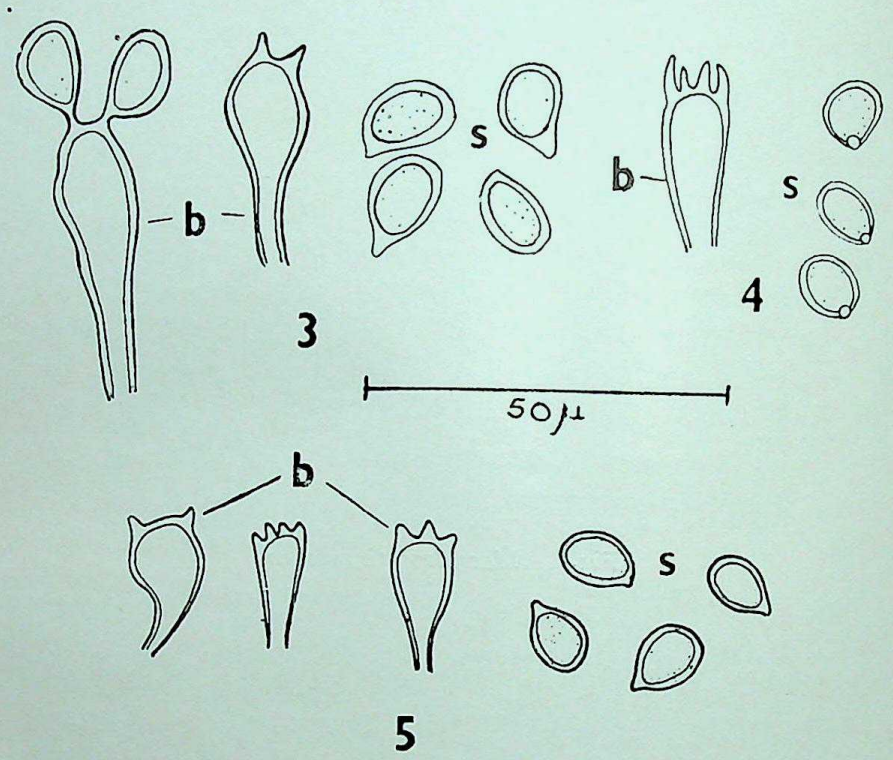


Fig. 2 *Macrolepiota rachodes*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 3– 5 Fig. 3 *Macrolepiota procera*. Fig. 4. *Macrolepiota rachodes*. Fig. 5. *Macrolepiota mastoidea*.

b=basidium. s=spores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the lamellae, pileus scaly but slightly smooth at the disc, annulus movable and double, volva absent. In this paper three species of *Macrolepiota*, viz., *M. procera*, *M. rachodes* and *M. mastoidea* are being described of which only *M. procera* has been listed in Butler and Bisby (1960), while *M. mastoides* has been listed as *Lepiota mastoidea* and *M. rachodes* as *Lepiotophyllum rachodes* for its white variety only i.e., *Lepiotophyllum rachodes* var. *puellaris*.

The materials have been deposited in the Herbarium, National Botanic Gardens, Lucknow (India).

Macrolepiota procera (Scop. ex Fr.) Singer *Pap. Mich. Acad. Sci.*, **32** : 141, 1946. (Syn. *Lepiota procera* Auct.) Figs. 1, 3.

Carpophores large, pileus 10-15 cm broad, slightly umbonate, grayish brown in colour, scaly on the disc, coarsely scaly elsewhere, more shaggy towards the edge, gills white turning slightly olivaceous on maturity, free, sub-remote, separated from the stipe by a large broad cartilaginous collar. Stipe 15-25 cm long, slender, slightly bulbous at the base, minutely scaly all over with sub-fuscous scales, hollow. Annulus large movable when dry. Flesh does not change colour on bruising. Spore print white, spores ovate, 13-16.5 × 8-10 μ, smooth with a germ pore.

Collected on soil, Lucknow, August 1963, N. C. Pathak, NBG No. 95709.

Macrolepiota rachodes (Vitt.) Singer *Lilloa*, **22** : 417, 1951.

(Syn. *Lepiota rachodes* Vitt., *Lepiotophyllum rachodes* (Vitt.) Locquin) Figs. 2, 4.

The species is rather more stunted than *Macrolepiota procera*. Pileus large, slightly convex, coarse, scaly rather a ragged pileus, 8-12 cm broad, cuticle of the cap breaking into coarse imbricate brown scales. Stipe 7-12 cm long, bulbous at the base and devoid of any scales. Gills white, closely connected to the stem by a white collar, free. Annulus large, movable when dry. Spores ovate, smooth, 8-10.5 × 5.5-6.5 μ.

Collected on soil, on waste ground, Lucknow, August 1963, R.N. Ghosh, NBG No. 94930.

The flesh of every part especially the stem turns red when bruised or cut.

Macrolepiota mastoidea (Fr.) Singer *Lilloa.*, **22** : 417, 1951.

(Syn. *Lepiota mastoidea* (Fr.) Quel.) Fig. 5.

Pileus 3-6 cm broad, white, cuticle breaking up into light gray to brown adpressed scales, campanulate, then convex and finally turning into acutely umbonate. Stipe 7-10 cm long, white squammulose.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

tough, attenuated at the apex, base bulbous. Annulus white, with brownish margin, movable. Gills white, free, remote, attached to the stem by a broad collar, soft. Spores elliptical, smooth, 13-14.5 × 8.5-10 μ.

Collected on soil, in fields, Lucknow, August 1963, R.N. Ghosh, NBG No. 94936.

Macrolepiota rachodes var. *puellaris* Fr.

This is almost half the size of the type species. Pileus about 5-6 cm broad, pure white, sometimes coarsely scaly. The flesh does not turn red on bruising. Spores oval, 8-10 × 5-6 μ.

Collected on soil near the fields, Lucknow, August 1963, N.C. Pathak, NBG No. 94929.

Summary

Three species of *Macrolepiota*, i.e., *M. procera*, *M. mastoidea*, *M. rachodes* and a variety of *M. rachodes*, *M. rachodes* var. *puellaris* have been described. *M. rachodes* is being recorded for the first time from India, while *M. rachodes* var. *puellaris* and *M. mastoidea* are new records for Uttar Pradesh. All the species of *Macrolepiota* are edible.

Acknowledgement: Authors are grateful to the Director, for his keen interest in this work. They are also grateful to the Head, Mycology and Plant Pathology Division, Indian Agricultural Research Institute New Delhi for help, Dr. Bahadur Singh for constant encouragement during the course of study and to Sri P. C. Roy for photographs.

Mycology Section,
National Botanic Gardens,
Lucknow (India).

References

Bresadola, G. (1927-41) *Iconographia Mycologica* Vol. **1-27** Mediolani.

Butler, E. J. and G.R. Bisby (1960) *Fungi of India*. Indian Council of Agricultural Research, New Delhi.

Hongo, T. (1959-60) *The Agaricales of Japan. Mem. Fac. Lib. Arts Shiga Univ.* **9** : 47-94 ; **10** : 61-72; *Act. Phyt.* **18** : 129-146.

Kaunrad, P. and A. Maublanc (1948) *Les Agaricales* I. *Agaricaceae. Enc. Mycol.* **14** : 1-469.

Kuhner, R. (1936) Recherches sur le genre *Lepiota. Bull. Soc. Myc. Fr.* **52** : 175-238.

Lange, J.E. (1935-40) *Flora Agaricina Danica*. Vol. **1-5**. Copenhagen.

Saccardo, P.A. (1887) *Sylloge Fungorum, V.*

Singer, R. (1951) *The Agaricales* (Mushrooms) *in Modern Taxonomy. Lilloa* **22** : 1-832.

——————— (1962) *The Agaricales in Modern Taxonomy*. J. Cramer, 1-915.

Sohi, H. S., S. Kumar and P. K. Seth (1964) Some interesting fleshy fungi from Himachal Pradesh. *Indian Phytopath.* **17** : 317-322.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MUTATION IN RACE 107 OF BROWN RUST OF WHEAT *PUCCINIA RECONDITA*

S. K. SHARMA AND D. P. MISRA*

(Accepted for publication September 17, 1965)

Rusts are generally stable in the clonal stage. However, there have been some records of pathogenic and colour mutations in the world [(Brown 1952), Newton and Johnson (1927, 39), Johnston (1930), Stakman *et al* (1930); Watson (1957) and others]. Of the two types of mutations, pathogenic ones have been less commonly reported. On the other hand, mutations in colour of uredia are distinguished readily. In India, Misra and Lele (1955) and Joshi and Kak (1955) reported the colour mutants in races 15-C and 194 of *Puccinia graminis* var. *tritici* respectively.

During the course of assay for races of *Puccinia recondita* Rob. ex. Desm. from 1962-63 crop in the country, a collection from Vijapur (Gujrat) on wheat variety E. 72, yielded race 107 which was reported by Mehta (1940). While recording the reactions on standard differential hosts, one pustule appeared distinctly different in colour in comparison with other normal pustules. Single spore culture from such an apparently abnormal pustule was established and designated as A. Another light brown coloured pustule was observed during the maintenance of race 107 in January 1964 after the race had completed about 400 uredospore generations. This culture is referred in these studies as B. The present papers deals with the morphologic and pathological characters of the single spore cultures of A and B in relation to type race 107.

OBSERVATIONS AND RESULTS: Uredospores of A are orange chrome and of B xanthine orange as compared to amber brown colour of type race. Uredinial pustules of A are slightly larger than those of type race and rupture almost normally, whereas, the uredinial pustules of B are slightly smaller than A and larger than type race, but these are more or less nonerumpant in comprison to the type race and culture A under similar conditions.

The mutants, however, were apparently weaker than the type race as the sporulation in both was comparatively less than that in the parent race. These also required longer incubation period than the type race. Under similar conditions at temperature ranges 12-24°C, the incubation period for A was 13 days ; for B, 14 days and for type race, 10 days. Under lower temperature ranges (7-13°C) the incubation period was more by 6-7 days in the mutants than in the type race.

*At Plant Pathological Sub-station, Indian Agricultural Research Institute, Simla-E.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

About one hundred uredospores from each of the three cultures were measured at random. Majority of the spores of cultures A and B were smaller than the type race, though the ranges for all the cultures were more or less overlapping. These characters are summarized in Table1.

TABLE 1

Culture	Colour of uredospore	Size of uredospores		Incubation period (12—24 °C)
		Range	Mean	
Parent race 107	Amber brown	17.6 to 26.8 μ × 16.6 to 23.4 μ	19.5 × 22.5 μ	10 days
Culture A	Orange chrome	17.2 to 26.8 μ × 15.3 to 23.0 μ	19.2 × 21.02 μ	13 days
Culture B	Xanthine orange	16.1 to 26.8 μ × 15.3 to 23.0 μ	18.9 × 20.5 μ	14 days

The three cultures were tested simultaneously on 25 wheat varieties besides the differential hosts (Johnston and Mains 1932), to ascertain the pathogenicity. While comparing the type race and the two mutants A and B, no difference was found on the differential hosts and 22 wheat varieties out of 25 tested. But on the remaining 3 varieties, the reactions were different as shown in Table 2.

TABLE 2 : Infection types produced by cultures A, B and type race 107 on 3 wheat varieties (symbols after Johnston and Mains 1932).

Sr. No.	Cultures	varieties		
		E. 5165	E. 5784	E. A. II. 5017
1.	Type race 107	0 ;	2-3	0 ;
2.	Culture A	3	2-3	2-3
3	Culture B	0 ;	0 ;	0 ;

As the above table shows the culture A is more virulent than culture B and the type race ; culture B is weakest in pathogenicity.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Since their isolation, the cultures A and B have been maintained at the Plant Pathological Sub-station, Indian Agricultural Research Institute, Simla on Agra local wheat. So far the cultures have remained stable for the characters reported.

DISCUSSION AND CONCLUSIONS : On standard set of differential varieties, cultures A and B produce reaction of race 107 of *Puccinia reconditia*, but differ from it and between themselves in pathogenicity on additional wheat varieties. Morphologically also, the two cultures are distinguishable. Physiologically, therefore, the two cultures are bio-types of race 107, CM-1 and CM-2.

With regard to the origin of 107 CM-1 various possibilities could be considered. This variation could not be due to infection from the alternate host, since the field collection was obtained from Vijapur (Gujrat), where the alternate host does not occur at all. Moreover in India, the alternate host has never been found to actually harbour the brown rust of wheat in nature (Mehta 1940). As a result of heterocaryosis in black rust of wheat (Nelson *et al* 1955), parent races of normal colour have not been found to produce the recombination of different colours. However, Bridgman (1959) observed that some new races which were produced through vegetative fusion of mycelium, differed from parent races in colour and pathogenicity, when he used uredospores of *Puccinia graminis tritici* races that were distinct in spore colour. In brown rust, there has been no record of a colour variant in India. In the light of these facts, the possibility of mutation or vegetative fusion appear to be suggestive in the origin of culture 107 CM-1. So far as culture 107 CM-2 is concerned, it was isolated from the type race 107, which has been maintained in the laboratory for about 28 years. This appears to be a clear instance of mutation.

SUMMARY

A light brown coloured uredinial pustule was isolated from a field collection of brown rust received from Vijapur (Gujrat), which was identified as race 107. A similar pustule appeared in the type race 107 after about 400 uredinial generations in the glass-house culture. These cultures are designated as 107 CM-1 and 107 CM-2, respectively. Both these cultures produce reactions similar to type race 107 on differential varieties but differ from it and between themselves in their colour and in pathogenicity on three additional wheat varieties. 107 CM-1 required about 3-5 days and 107 CM-2, 4-6 days more than the parent race for full development of uredospores.

ACKNOWLEDGEMENTS : Authors are grateful to Dr. R. Prasada, Mycologist, Indian Agricultural Research Institute, New Delhi for encouragement and his keen interest during the course of these studies.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

BRIDGMAN, G. H. (1959) Production of new races of *Puccinia graminis* var. *tritici* by vegetative fusion. *Phytopathology* 49 : 386-388.

BROWN, A. M. (1952) Mutation in incitant of leaf rust of wheat *Puccinia triticina*. *Phyopathology* **42** : 479.

JOHNSTON, C. O. (1930) An aberrant physiologic form of *Puccinia triticina*. Erikss. *Phytopathology* **20** : 609-620.

JOHNSTON, C. O. AND E. B. MAINS (1932) Studies on physiologic specialization in *Puccinia triticina*. Dept. of Agric. Tech. Bull. 313.

JOSHI, L. M. AND D. KAK (1955) Mutation in *Puccinia graminis tritici* (Pers.) Erikss. and Henn., Physiologic race-194. *Indian Phytopath.* **7** : 202-204.

MEHTA, K. C. (1940) Further studies on cereal rusts in India. Sci. Monogr. No. 14. *Ind. Coun. Agric. Res.* India. 240 pp.

MISRA, D. P. AND V. C. LELE (1955) Mutation in *Puccinia graminis tritici* (Pers.) Erikss & Henn. Physiologic race 15-C. *Indian Phytopath.* **7** : 79-81.

NELSON, R. R., R. D. WILCOXSON and J. J. CHRISTENSEN (1955) Heterocaryosis as a basis for variation in *Puccinia graminis tritici*. *Phytopathology* **45** : 639-643.

NEWTON, M. AND T. JOHNSON (1927) Colour mutation in *Puccinia graminis tritici* (Pers.) Erikss and Henn. *Phytopathology* **17** : 711-725.

———,——— (1939) A mutation for pathogenicity in *Puccinia graminis tritic*. *Can J. Res* (C) **17** : 297-299.

STAKMAN, E. C., M. N. LEVINE AND R. U. COTTER. (1930) Origin of physiologic forms of *Puccinia graminis tritici* through hybridization and mutation. *Sci. Agric.* **10** : 707-720.

WATSON, I. A. (1957) Mutation for increased pathogenicity in *Puccinia graminis tritici* *Phytopathology* **47** : 507-509.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MYCOSTATIN FOR PREVENTION OF ANTHRACNOSE OF BANANAS IN STORAGE

ANDAL ANANTHANARAYANAN AND K. SESHADRI

(Accepted for Publication November 3, 1965)

Anthracnose caused by the fungus *Gloeosporium musarum* Cke. and Massee destroys many bananas in transit, storage and market. It occurs on all varieties of bananas. The infection becomes evident mostly when yellow colour develops in the fruit and may cause blemishes or complete decay of the fruit. Consequently, serious losses occur, especially in shipments during transit for marketing in other countries. Meredith (1960 a, b) reported that Nystatin (also referred to as Mycostatin or Fungicidin), when applied as Post-inoculation fruit dip at a concentration of 200-400 ppm., effected good control of wound infection by *G. musarum*. The percentage control was inversely related to the incubation period between inoculation and treatment, there being little or no control with periods exceeding 30 hours. Rangaswamy and Damodaran (1957) found that Mycostatin had a high fungicidal and fungistatic activity against *G. musarum*, *in vitro*. Damodaran (1961) found that among several fungicides and antibiotics tested, Bordeaux mixture 1 per cent, Griseofulvin 0.1 per cent, and Mycostatin 100 ppm. effectively controlled anthracnose when used as a prophylactic treatment 20 hours prior to inoculation but none of them were effective as a curative, except Bordeaux mixture 1 per cent which gave slight control. With the object of testing the effect of Mycostatin treatment on development af anthracnose in fruits of different varieties of banana, and the duration and persistence of the antifungal activity of the antibiotic, the present investigation was carried out and the results are presented here.

MATERIAL AND METHODS: A sample of pharmaceutical grade of Mycostatin was obtained from the Squibb Institute, New Brunswic, N. J., U. S. A. through the Squibb Sarabhai Ltd., Ahmadabad, and used throughout the investigation. The antibiotic was used at a concentration of 400 ppm. (1. 52 g. of Mycostatin was dissolved in 190 ml. of rectified spirit and diluted to one gallon. Since the solvent Dimethyl formamide, recommended by the firm was not available, rectified spirit was used). For inoculation, the fruits were dipped in a spore suspension of an isolate of *G. musarum* from bananas collected at Yercaud, Salem district. For curative treatment, the fruits were first dipped in spore suspension and then dipped in Mycostatin, and *vice versa* in case of prophylactic treatment. The fruits were then placed in alkathene bags and openings of the bags were sealed with wax to prevent contamination.

EXPERIMENTAL: Three laboratory experiments were conducted to find out the effect of Mycostatin on various varieties of banana. In the first experiment mature but green fruits of the starchy variety, Monthan, were used. There were five treatments and four replications with three

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

fruits per replicate. The particulars regarding the treatments and results are presented in Table 1. The incubation period required for the development of symptoms was 23 days.

TABLE 1 : The effect of Mycostatin on development of anthracnose on variety Monthan.

No.	Treatment	No. of fruits showing symptoms out of three				Remarks
		Rep. I	Rep. II	Rep. III	Rep. IV	
1.	Treated with Mycostatin 24 hours prior to inoculation.	3 (I)*	Nil	Nil	2 (I)	All fruits were partly ripe.
2.	Inoculated and 24 hours later treated with Mycostatin.	3 (III)	3 (III)	3 (III)	3 (III)	
3.	Treated with Mycostatin alone without inoculation.	Nil	Nil	Nil	Nil	
4.	Inoculated but not treated with Mycostatin.	3 (I)	3 (II)	3 (III)	3 (III)	
5.	Control (untreated)	Nil	3 (I)	3 (I)	3 (II)	All fruits were ripe.

*The figures within brackets show symptom grade. 'I'=very few small spots on fruits ; 'II'—few large spots covering nearly ¼ of the fruit ; 'III'=numerous spots covering the entire fruit.

From the above results, it will be seen that the antibiotic treatment 24 hours prior to inoculation has prevented the development of anthracnose to a large extent. The treatment with antibiotic 24 hours after inoculation has not given any control.

The variety Kaali was used in the second experiment and the duration of the persistance of the effect of antibiotic was also tested. There were seven treatments, with four replicates per treatment ; and two fruits per replicate. The other experimental details were same as mentioned earlier. The bags with fruits were kept in a wooden box and incubated under laboratory conditions. The results of the experiment are presented in Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Effect of Mycostatin on development of anthracnose on variety Kaali.

No.	Treatment	No. of fruits showing symptoms out of 2 per replicate			
		Rep. I	Rep. II	Rep. III	Rep IV
1.	Treated with Mycostatin 24 hours prior to inoculation.	Nil	Nil	Nil	Nil Slight blemish in one fruit.
2.	Treated with Mycostatin 48 hours prior to inoculation.	Nil	Nil	1 (I)	1 (I)
3.	Treated with Mycostatin 96 hours prior to inoculation.	Nil	Nil	Nil	1 (I)
4.	Treated with Mycostatin 24 hours after inoculation.	2 (III)	2 (II)	2 (II)	2 (II)
5.	Treated with Mycostatin alone without inoculation.	Nil	Nil	Nil	Nil
6.	Inoculated but not treated with Mycostatin.	2 (III)	2 (III)	2 (III)	2 (III)
7.	Control (untreated)	Nil	Nil	Nil	Nil

The results show that the antibiotic prevented completely the development of anthracnose when used as a prophylactic treatment 24 hours prior to inoculation and prevented the development of the disease to a very great extent even after 96 hours after treatment with the antibiotic.

In the third experiment, the effect of water suspension of Mycostatin (without alcohol) was also studied (treatment No. 4 in Table 3). The variety used was Nendrapadathi. The results of the experiment are presented in Table 3.

A dip in Mycostatin inhibited the development of anthracnose in this case also. Antibiotic treatment 72 or 120 hours prior to inoculation had reduced the severity of symptoms to a great extent. There was not much difference between antibiotic dissolved in alcohol then diluted with water and the one in which it was directly used as water suspension.

The complete inhibitory activity of the antibiotic was also confirmed by testing spore germination in antibiotic solution and the growth of the fungus from the peels of the fruits treated with antibiotic.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Effect of Mycostatin on development of anthracnose in the variety Nendrapadathi.

No.	Treatments	No. of fruits showing symptoms out of 2 per replicate.			
		Rep. I	Rep. II	Rep. III	Rep. IV
1.	Treated with Mycostatin 24 hours prior to inoculation.	Slight blemish in one.	Nil	Nil	Nil
2.	Treated with Mycostatin 72 hours prior to inoculation.	2 (I & II)	2 (I)	2 (I)	2 (I)
3.	Treated with Mycostatin 120 hours prior to inoculation.	2 (I)	2 (I)	2 (I & II)	2 (I)
4.	Treated with Mycostatin as suspension in water 24 hours prior to inoculation.	Nil	Slight blemish in one.	Nil	Slight blemish in one.
5.	Treatment with Mycostatin alone without inoculation.	Nil	Nil	Nil	Nil
6.	Inoculated but not treated with Mycostatin.	2 (III)	2 (III)	2 (III)	2 (III)

Inhibition of fungal growth from peels of Mycostatin treated fruits :

Samples of peels from fruits that were (i) treated with Mycostatin and then inoculated, and (ii) those that were only inoculated without the antibiotic treatment, were plated in oats agar after two days incubation. Twelve bits were plated from each sample. After five days there was growth of *Gloeosporium*, only from the peels of fruits which were inoculated but did not receive antibiotic treatment, whereas there was no growth from peel samples of fruits treated with antibiotic before inoculation.

Inhibition of fungal spore germination :

Spores of *G. musarum* were suspended in sterile tap water and in 400 ppm. of the antibiotic in cavity slides in moist chambers. After 24 hours, there was 90 per cent germination in sterile water, whereas in antibiotic there was compelte inhibition of germination. In another experiment, alcohol diluted with water in the same concentration used in preparing Mycostatin solution, was tested (without the antibiotic) for its effect on

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

spore germination. There was no inhibition of germination, showing that the fungicidal effect of the solution was entirely due to Mycostatin and not due to alcohol in the solution.

DISCUSSION : Mycostatin has been found effective in the control of storage rot of bananas. It seems to act as a protetcant and disinfectant, but not as a therapeutant.

Inhibition was most effective when prophylactic treatment was given 24 hours prior to inoculation with fungal spore suspension. The severity of the symptoms increased as the interval between prophylactic treatment and inoculation with fungal spores increased, so that after 72 to 120 hours, the control was only partial, though the antibiotic retained considerable activity even after 120 hours. It would therefore be desirable, that to obtain maximum effect of Mycostatin, the fruits after prophylactic treatment may prormptly be sealed in alkathene bags. Fruits that were treated with Mycostatin and placed in alkathene bags, were found to be completely blemish free throughout the period of experimentation, i. e., ten days in case of Kaali and Nendrapadathi, and 23 days in case of Monthan. This indicates the possibility of obtaining blemish free fruits even if stored for considerably long time in absence of refrigeration. Bagging done promptly after treatment will not only minimise further fresh infection, but will also decrease wounds and bruises caused during handling. According to Meredith (1960 b) bagging had no significant effect on inducing rotting. Rather it was the presence of perforated polythene bags that favoured development of disease.

There was not much difference between antibioiic dissolved in alcohol (and then) diluted with water and the one in which it was mixed directly in water as a suspension, in the concentration tested.

SUMMARY

The effect of Mycostatin on storage rot of bananas caused by *Gloeosporium musarum* Cke. and Massee was tested on three varieties of bananas. The antibiotic was effective as a prophylactic than as a therapeutant, especially when the prophylactic treatment was given 24 hours prior to inoculation, though the antifungal activity was perceptible even up to 120 hours after treatment. Fungicidal activity of Mycostatin was also confirmed by spore germination tests, and the absence of fungal growth in peels of fruits treated with antibiotic before inoculation, plated on oat agar.

ACKNOWLEDGEMENT : The authors express their deep sense of gratitude to Dr. K. Ramakrishnan, Associate Dean, for his kind help and guidance in the preparation of this paper. Thanks are also due to Sri C. S. Krishnamurthy, Mycologist and Associate Professor of Plant Pathology, for his interest and encouragement during the work. The authors are indebted to the squibb Institute, U. S., and M/S Squibb Sarabhai Ltd., for the sample of Mycostatin supplied gratis. Grateful acknowledgement is due to the Indian Council of Agricultural Research

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

for providing a grant to support the scheme for investigation of Bananas in which this item of work was done.

Section of Mycology,
Agricultural College and Research Institute,
Coimbatore-3

REFERENCES

DAMODARAN, A. P. S. (1961) *Studies on anthracnose of bananas*. A thesis submitted to the University of Madras for the award of degree of Master of Science.

MEREDITH, D. S. (1960a) Studies on *Gloeosporium musarum* Cke. and Massee causing storage rots of Jamaican bananas. I. Anthracnose and its chemical control. *Ann. Appl. Biol.* **48** : 279-290.

————, (1960b) Studies on *Gloeosporium musarum* Cke. and Massee causing storage rots of Jamaican bananas. II. Some factor influencing anthracnose development. *Ann. appl. Biol.* **48** : 518-528.

RANGASWAMY, G. AND A. P. S. DAMODARAN, (1957) *In vitro* action of Mycostatin on fungi pathogenic to bananas—*Indian Phytopath.* **10** : 126-129.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FURTHER STUDIES ON CHIRKE DISEASE OF LARGE CARDAMOM (AMOMUM SUBULATUM ROXB.)

S. P. RAYCHAUDHURI[1] AND B. GANGULY

(Accepted for publication December 21, 1965)

The large cardamom suffers from 'Chirke', a virus disease. The leaves of affected plants exhibit mosaic streaks (Fig. 1), which gradually coalesce and eventually turn brownish and dry up. The flowering in diseased plants is extremely reduced and only 1 to 5 flowers develop in one inflorescence as against 16 to 20 flowers borne by healthy plants (Raychaudhuri and Chatterjee, 1958 ; 1961) and hence the yield is very much reduced. The virus is sap-transmissible and also spreads by the agency of the aphids, *Rhopalosiphum maidis* Fitch. (Raychaudhuri and Chatterjee, 1958 ; 1961 ; 1964) as well as by *Brachycaudus helichrysi* Kalt. (Raychaudhuri and Chatterjee, 1965).

Work on physical properties and some tests on varietal resistance of large cardamom and wheat have been reported earlier (Raychaudhuri and Chatterjee, 1961). The present paper deals with further studies on varietal resistance of large cardamom and wheat, assessment of losses and serological studies. It also describes the progress made to cure the diseased plants by both heat and chemotherapeutic treatments.

MATERIAL AND METHODS : Young actively growing plants were inoculated in insect-proof cages mechanically, using carborundum as an abrasive.

For preparing antiserum, the diseased leaf extract was clarified by heating for 10 minutes at 45°C, followed by immediate cooling and centrifugation, to remove the bulky plant material. To the clarified sap, saturated solution of ammonium sulphate (made by adding 760 gms. of the salt for each litre of distilled water) equivalent to half the volume of the sap was added to precipitate the virus, followed by further centrifugation. The sediment was dissolved in a small quantity of distilled water and dialysed. One c. c. of this partially purified virus was then injected in the marginal vein of the ear of an albino rabbit twice weekly with a hypodermic syringe. To ensure better antisera 2 c.c. of infective sap in addition was also injected intraperitoneally along with every intravenous injection. In all ten injections were administered in a single rabbit. The rabbit was then bled 10 days after the last injection and the blood allowed to clot and the serum separated, centrifuged and preserved by addition of a few drops of chloroform and kept in a cool place. The normal serum was obtained from the animal which had never been previously injected with any antigenic material.

Rapid agglutination tests were made by squeezing out two drops of leaf extract from the suspected plant on a glass slide. A drop of the antiserum was added to one of the drops and a drop of the normal serum

[1]Present address : Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

to the other. The material was stirred with opposite ends of a tooth-pick. Agglutination of chloroplasts with the antiserum indicated the presence of the virus.

Assessment of losses was made on 100 plant basis. One year old artificially infected plants were used and plants of the same age and height were selected for experimental purpose. Data on the production of flowers, fruits and seeds were recorded.

RESULTS AND DISCUSSION : The virus was transmitted mechanically to the large cardamom varieties Kati, Hario and Churamfa. Variety Kopringe proved to be resistant to this disease. This is the second resistant variety in addition to Sawney reported earlier (Raychaudhuri and Chatterjee, 1961).

Twenty wheat varieties viz., N. P. 770, N. P. 792, N. P. 824, N. P. 829, N. P. 849, H. B. 6-61-14, H. B. 661-18, H. B. (d) 389-58-308, H. P. 101-469, H. P. 330-64, H. P. 136-61-18, H. S. 636-58-274, H. S. 701-58-280, H. S. (d) 167-7-8-3-4, H. S. (d) 389-10-5-4, H. S. (d) 637-58-280, H.S. (d) 167-8-4-2-6-6, H. S. (d) 289, K 64 and K 65 were found to be susceptible to the virus, while the variety Ridley proved to be highly tolerant to the virus and varieties N.P. 803 and 809 were moderately resistant. In addition, 11 promising selections of ginger viz., Bonglong, Bijanbari, Rohini, Tagdah, Dongra, Algarah, Nim, Dallapchand, Naya Bazar, Sorang and Kalimpong Local and a perennial weed, *Acorus calamus* L. also proved to be susceptible. Both wheat and ginger are commonly grown in Kalimpong region and may act as collateral hosts in spreading the disease.

Considering that the disease is transmitted in nature through the agency of aphids, it is important to trace the diseased plants in the field as early as possible and rogue them out before it spreads to other plants. A rapid serological method was developed to identify the diseased plants in the field (Ganguly, 1965). Rapid chloroplast agglutination tests were made to locate the diseased plants under the field conditions. The infected plants after detection can be rogued out thereby checking the further spread of the virus. Using this technique Chirke virus was detected in 1,193 and 1,828 plants out of 16,012 and 20,385 plants tested serologically in 1963 and 1964, respectively from the different cultivators' plots of the Darjeeling district.

Studies on assessment of losses were continued for three years from 1962 to 1964. Table 1 shows the comparative yearly yield data in respect of healthy and diseased plants.

It is evident that the diseased large cardamom plants developed less number of flowers, fruits, and seeds, as compared to healthy plants of the same age, the percentage loss being 85.20 and 80.09 in total number of fruits and seeds respectively, by the end of third year of the crop.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 1. Symptoms of Chirke disease of large cardamom

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Assessment of losses due to Chirke disease of large cardamom during 1962-1964

Year	Healthy (out of 100 clumps)				Diseased (out of 100 clumps)				Loss in percentage			
	Total No. of		Total weight of (in gms.)		Total No. of		Total weight of (in gms.)		No. of Fruits	No. of Seeds	Wt. of Fruits	Wt. of Seeds
	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds	Fruits	Seeds				
1962	193	1200	82.0	—	192	1162	78.0	—	0.51	3.16	4.87	—
1963	1156	16113	2715.7	449.5	546	4973	867.2	193.3	52.76	69.13	68.06	56.99
1964	1602	24909	3438.6	651.9	237	2958	115.5	64.8	85.20	80.09	96.64	88.12

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The above data prove that there is a progressive degeneration of the diseased stocks from year to year and hence it requires thorough roguing of the diseased plants in time and replenishment with certified virus-free seedlings.

Preliminary test on the effect of heat-therapy to cure the disease revealed that dry heat treatments of chirke affected rhizomes at 30°, 32°, 34°, 36°, 38°, and 40°C. for 2, 4, 6, 8, 10 and 12 hours could not cure the disease. Chemo-therapeutic tests on the effect of soil drenching of large cardamom plants by thiouracil (2-mercapto-4-hydroxy pyrimidine) at 0.1 per cent before or at the time of inoculation indicated beneficial effects and the test plants remained healthy (Raychaudhuri and Chatterjee, 1961) while dip treatments at 0.001 per cent, 0.0001 per cent and 0.00001 per cent solution of propionic acid, follic acid, 2-amino-n-butyric acid, DL-norleucine, taurine, benzimedazole, colchicine, sulphonalimide, DL-ethionine, phenyl acetic acid alphanaphthalene acetic acid, indole-3-butyric acid and azaguanine for 3, 6, 9, 12 and 24 hours and 10, 15, 20, 30, 50 and 100 p. p. m. of malachite green for 3, 6, 9, 12, 16, 24, 36, 48 and 72 hours failed to cure the disease. However, when treatments were given with hydroquinone at concentrations ranging from 0.001 per cent to 0.1 per cent, promising signs of inhibition of the virus could be detected in the infected rhizomes. Those which were treated at 0.075 per cent and above, with this chemical, did not show any symptoms and appeared healthy. Back inoculations using the juice extracted from the youngest leaves of recovered plants indicated negative results.

The virus under study resembles the virus infecting saccharine sorghum, grain sorghum and maize (Lovisolo, 1956) in physical properties and the vector, but differs in its host range (Raychaudhuri and Chatterjee, 1961).

SUMMARY

Varietal and host-range studies of the virus causing chirke disease of large cardamom (*Amomum subulatum* Roxb.) proved that the variety Kopringe is resistant to this virus in addition to the variety Sawney reported earlier. Twenty wheat varieties proved to be susceptible to this disease while the varieties Ridley, N. P. 803 and N. P. 809 proved to be tolerant to it. Besides, 11 promising varieties of ginger and the perennial weed, *Acorus calamus* L. were found to be susceptible to this virus.

Antiserum was prepared against this virus. Rapid agglutination test proved that 3,021 out of 36,397 plants tested from different cultivators' fields in Darjeeling district were infected with the virus.

It was experimentally proved that the losses are as high as 85.20 and 80.09 per cent in total number of fruits and seeds respectively, through degeneration due to this virus after three years.

Preliminary tests on the effect of heat-and chemo-therapy of the virus revealed that dry heat treatments at temperatures ranging between 30°C to 40°C and treatments with 14 chemicals consisting of growth regulators, antimetabolites and other organic compounds for varying

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

periods failed to cure the disease while promising signs of inhibition of the virus were observed when infected rhizomes were given 0.075 per cent and above treatments with hydroquinone besides soil drenching with 0.1 per cent of thiouracil, reported earlier.

Indian Agricultural Research Institute,
Plant Virus Research Substation,
Eastern Zone, Kalimpong.

References

GANGULY, B. (1965) A rapid test for detecting chirke affected large cardamom (*Amomum subulatum* Roxb.) plants in field. *Sci. and Cult.* (In press).

LOVISOLO, O. (1956) Contribute aperimentale alla conoscenze ed alla teterminazione del virus agente dell' arrosamento striato del sergo e di un mosaico del mais. *Bollentino della stazione di Potalogia Vegetale*, **14** : 261-321.

RAYCHAUDHURI, S. P. AND S. N. CHATTERJEE (1958) A preliminary note on the occurence of a new virus disease of large cardamom (*Amomum subulatum* Roxb.) in Darjeeling district. *Mycological Research Workers' conference*, Simla 1958.

RAYCHAUDHURI, S. P. AND S. N. CHATTERJEE (1961) Chirke a new virus threat to cardamom. *Ind. Farm*, **9** : 11-12.

RAYCHAUDHURI, S. P. AND S. N. CHATTERJEE (1964) Aphid transmission of a new mosaic streak disease of large cardamom. *Phytopathology* (Abs.) **54** : 904.

RAYCHAUDHURI, S. P. AND S. N. CHATTERJEE (1955) Transmission of chirke disease of large cardamom by *Aphid* species. *Indian J. Entom.* **27** : 272-276.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

Effect of fungicides on the viability of Brassica seeds—S. B. MATHUR, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—Vegetable seeds lose their viability rapidly on storage. If the seeds can be stored for a longer period without losing the viability, the requirement of land for seed production can be reduced and land can be used for other purposes. The effect of seed-dressing fungicides on the viability of some brassica seeds stored in glass bottles with backolite caps for a period of one year under room temperature conditions (i. e. 15-30°C) was therefore determined. Pritchard' have reported that the seeds of carrot, cauliflower, lettuce and onion lost germination rapidly while tomato seeds retained their viability very well. Clayton[2] reported that the treatment of vegetable seeds with mercuric chloride affected their viability.

The vegetable seeds of cauliflower (Pusa Katki), cabbage (Golden Acre), Radish (White Icicle) and Turnip (Golden Ball) were treated with Agrosan GN (phenylmercury acetate and ethylmercury chloride), Semesan [2 chloro—4 (hydroxy mercuri) phenol], Spergon (tetrachloro-p-benzoquinone), Thiram [bis (dimethyl thiocarbamoyl) disulphide], and Captan [n-(tri-chloromethyl mercapto) 4-cyclohexene- 1, 2, dicarboximide.] on a mechanical shaker to give uniform coating of fungicides on the seeds. Untreated seeds stored under similar conditions served as control. The moisture content of cauliflower, Cabbage, Turnip and Radish seeds before storage was 10.6, 11.2, 11.5 and 10.8 per cent respectively and 7.5, 7.1, 6.2 and 6.0 per cent after one year storage at room temperature. Four replications were used throughout the present investigation. The data regarding effect of fungicides on germination of seeds are summarised in Table 1.

TABLE 1 : Viability of Fungicide treated seeds.

Fungicide	Dosage per cent	Percentage germination of seeds							
		Cauliflower		Cabbage		Radish		Turnip	
		B	A	B	A	B	A	B	A
Agrosan GN	0.3	100.0	95.0	66.0	10.0	41.0	20.0	92.0	27.0
Semesan	0.25	96.0	87.0	53.0	4.0	57.0	29.0	93.0	39.0
Spergon	0.5	98.0	83.0	43.0	2.0	43.0	16.0	92.0	31.0
Thiram	0.3	97.0	84.0	47.0	2.0	40.0	14.0	91.0	21.0
Captan	0.3	95.0	84.0	53.0	3.0	52.0	13.0	85.0	32.0
Control (Untreated)	—	98.0	83.0	58.0	5.0	47.0	16.0	83.0	31.0

Note : 'B' denotes the percentage germination before storage and 'A' denotes the percentage germination after storage of one year.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It is evident from the data in table I that Agrosan GN can be recommended for seed treatment of cauliflower and cabbage, Semesan for treatment of Radish and Turnip seeds.

ACKNOWLEDGEMENT : The author is indebted to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi and to Dr. J.S. Grewal for encouragement during the course of this work.

[1]Pritchard, E. W. *J. Agric. Soc.*, **72** : 168-83, (1911).
[2]Clayton, E.E. *Phytopath.* **21** : 105-106, (1931).

———

Effect of Zinc and Amino acids* on the growth of Fusarium oxysporum f. cumini Prasad and Patel.—B. L. MATHUR AND H. C. SANKHLA, Plant Pathology Section, Rajasthan, Jaipur.—Agarwal (1959) and Saraswati Devi (1955) reported that zinc was indispensable for normal growth of various species of *Fusarium*. Saraswati Devi (1958) further reported that from poor growth in the zinc omitted cultures, the organism steadily improved uptill it reached the sufficiency level, and normal growth was attained. Further increase in zinc supply appeared neither to increase nor decrease the amount of growth to any considerable extent.

Single amino acids or mixtures of amino acids are often used as sources of nitrogen for the growth of fungi in culture. The percentage of carbon in individual amino acids varies, leucine contains 55 per cent, where as alanine contains only 32 per cent. The contribution of the amino acids carbon to the total carbon content of media frequently appears to be neglected.

In the present investigation the effect of zinc on the growth and utilization of amino acids as sole source of carbon by *Fusarium oxysporum f. cumini* were studied. The experiments were carried out with six isolates of the fungus. (Mathur and Prasad, 1964). The Richard's medium was used as the basal medium. The medium was purified by following the process of Steinberg and then $FeCl_3$ 0.02 per cent was added. Zinc as zinc sulphate was added to the medium in different concentrations upto 100 ppm per litre.

The carbon and nitrogen sources were deleted and amino acids added containing 4.8 gms of carbon per litre of basal medium, glucose carbon with potassium nitrate was kept as control.

Zinc when added in trace amounts (5 ppm) the growth increased in all the isolates. Addition of zinc up to 50 ppm appeared ineffective on the growth of isolates 1 and 33 ; suppressed the growth of isolates 4, 40 and 41 ; where as increased the growth of isolate 27. Further supply upto 100 ppm inhibited the growth of all isolates except 27 and 33, where it increased the growth. Isolate 33 showed poor growth in presence of vitamins, Mathur *et. al.* (1964) ; but the growth was stimulated markedly on addition of zinc and the growth was almost equal to that of other isolates.

**Note* : The experiments were carried out by using agar (Difco) medium and the possibilities of there being traces of carbon sources are not ruled out.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Effect of zinc on mycelial weight of *Fusarium*.

Zinc concentration in ppm	Mycelial weight in mg./20 ml. media after 10 days					
	Isolate 1	Isolate 4	Isolate 27	Isolate 33	Isolate 40	Isolate 41
0	135.2	332.9	129.9	62.5	176.3	241.1
5	292.5	400.9	247.9	206.9	270.6	323.3
15	297.5	407.5	247.4	205.3	271.7	334.6
25	304.8	412.4	244.7	201.5	276.9	341.6
50	301.1	406.4	254.1	200.4	262.7	323.9
100	295.6	397.1	265.0	243.6	230.0	318.1

Five isolates could utilize carbon and nitrogen as the sole source from B-alanine, dl-asparatic acid, l-asparagin, l-leucine, l-glutamic acid. Isolate 27 could not utilize any of the amino acids as carbon and nitrogen source. None of the isolates made appreciable growth on creatine, methionine and dl-norvaline. In another experiment amino acid-carbon was supplemented with glucose carbon and it was observed that the growth in amino acid-carbon combined with glucose-carbon was better than that of amino acid-carbon and glucose singly.

Treggi (1954) investigated the ability of five pathogenic fungi to grow on media in which single amino acid served as the sole source of nitrogen and carbon. All the fungi tested grew well on asparatic acid. In addition *Fusarium lini* Bolley grew well on leucine. None of the five species made appreciable growth on glycine and threonine. Lilly and Barnett (1956) reported that *Phycomyces blakesleeanus* Burgeff and *Schizophyllum commune* Fr. utilized the carbon in casein hydrolysate poorly, yet in presence of glucose good growth ensued. The results of present studies show that it is important to distinguish between the value of amino acid as a source of carbon and nitrogen.

Grateful thanks are due to Dr. R. L. Mathur, Plant Pathologist, Rajasthan, Jaipur for encouragement and the Director of Agriculture, Rajasthan, Jaipur for providing laboratory facilities.

[1]Agarwal, G. P. *Phyton* **12** : 2, 87-91 (1959).

[2]Lilly, V. G. and H. L. Barnett. *Proc. West. V. Acad. Sci.* **28** : 20-24 (1956).

[3]Mathur, B. L. and N. Prasad. *Ind. Jour. Agri. Sci.* **34** : 131-137 (1964).

[4]Mathur, B. L., L. C. Sharma and N. Prasad. *Indian Phytopath.* **17** : 121-125 (1964),

[5]Saraswati Devi. *Proc. Indian Acad. Sci.* **41 B** : 145-150. (1955).

[6]————————. *Jour. Indian Bot. Soc.* **37** : 509-516 (1958).

[7]Steinberg, R. A. *J. Agric. Res.* **51** : 412-424 (1935).

[8]Treggi, G. *Ann. sper. agrar* **8** : 1955-63 (1954).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Utilization of Growth Regulators by Fusarium oxysporum f. cumini Prasad and Patel.—H. C. SANKHLA, B. L. MATHUR AND R. L. MATHUR, Plant Pathology Section, Department of Agriculture, Rajasthan, Jaipur.—The quantum of research work conducted with respect to utilization and essentiality of growth regulating substances by fungi appears fragmentary, when compared to the extensive studies carried out on other aspects of fungal physiology and the influence of auxins and anti-auxins on the multifarious activities of higher plants. Leonian and Lilly[2] working with about 100 fungi, Richard[5] with 4 fungi, Benerjee and Mukhopadhaya[1] with *Merulius similis* B. and B. R. and Misra and Mahmood[4] with *Colletotrichum capsici* (Sydow) Butler and Bisby obtained beneficial effects of addition of lower concentrations of growth regulating substances (hormones) to the media, but higher concentrations were found inhibitory. Mehrotra[3] however, observed that hormones were valueless for stimulation of growth of *Phytophthora spp.* The effect of five concentrations of five growth regulators on isolate No. 1 of *Fusarium oxysporum f. cumini* Prasad and Patel responsible for wilt disease of Cumin (*Cuminum cyminum* L.) was studied and the results are described in this paper.

Five growth regulators namely Maleic hydrazide (MH), 1—Naphthyl acetic acid (NAA), 2—Naphthoxy acetic acid (NoAA), Indole-3-yl-acetic acid (IAA) and Indolyl-3- butyricum acetic acid (IBA) were incorporated in Richard's medium, so as to give concentrations of 0.05, 0.5, 1.0, 5.0, and 50.0 parts per million. Pyrex flasks of 100 ml. were filled with 25 ml. solutions, sterilized at 15 lb. pressure for 10 minutes and the solutions inoculated with single germinating conidium of the fungus under study in three replications. The mycelial mats of the fungus were harvested after 13 days by filteration on weighed whatman paper, washed thoroughly, dried, reweighed and the dry weight calculated. The observations are recorded in the following table.

TABLE : Average dry mycelial weight of Cumin wilt *Fusarium* on Richard's medium with different concentrations of growth regulators.

S. No.	Growth regulators	Concentrations	Weight of dry mycelium in mg.					
			0.0	0.05	0.5	1.0	5.0	50.0
1.	MH	—	—	167	203	172	147	121
2.	NAA		—	158	155	134	108	77
3.	NoAA		—	92	108	115	96	81
4.	IAA		—	95	103	121	88	66
5.	IBA		—	94	100	108	88	62
6.	Control		90	—	—	—	—	—

Critical difference = 36.6 at 5 per cent.
" " = 50.8 at 1 per cent.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results indicate that all the chemicals resulted in better mycelial growth of the fungus upto 1.0 ppm concentrations ; only IAA and IBA at 5 ppm and all the chemicals except MH at 50 ppm reduced the weight to a certain extent. None of the regulators proved toxic upto the limit of concentrations tested. Statistical analysis of the data revealed significant differences between the treatments at 1 per cent level. Maleic hydrazide at all concentrations except 50 ppm and 1—naphthyl acetic acid at 0.05, 0.5 ppm gave significantly higher yields of mycelium over control at 1 per cent level, one ppm of NAA proving beneficial at 5 per cent level of significance only. None of the other concentrations of the chemicals used could either significantly increase or decrease the mycelial growth.

The cumin wilt fungus behaved like other fungi studied by earlier workers except Mehrotra (1951) in that the tested growth regulating chemicals in general proved beneficial for its growth in lower concentrations. With higher concentrations there were indications of inhibitory effect though statistically significant differences were not observed.

The authors wish to express their thanks to Shri T. C. Kala, Director of Agriculture, Rajasthan, Jaipur for providing necessary facilities.

[1]Benerjee, S. and S. Mukhopadhaya. *Indian J. Mycol. Res.* **3** : 59-63 (1957).

[2]Leonian L. H. and V. G. Lilly *Amer. J. Bot.* **24** : 135-39 (1937).

[3]Mehrotra, B. S. *Curr. Sci.* **20** : 131-132 (1951).

[4]Misra, A. P. and M. Mahmood, *Indian Phytopath.* **14** : 20-22 (1961).

[5]Richard R. R. *Bot. Gaz.* **110** : 523-550 (1949).

———

A virulent race of Puccinia striiformis West. on barley and sources for its resistance. —SHEODHAN SINGH, S. T. AHMAD AND D. P. MISRA, Indian Agricultural Research Institute, Plant Pathological Sub-station, Flowerdale, Simla. —In the routine analysis of yellow rust collections for race determination during the crop season 1963-64, a sample from barley variety NP. 108 received from Wheat Breeding Sub-station, Wellington (Nilgiris), Madras state, produced infection type on yellow rust differentials similar to race 24 (Table I) which was originally found on barley in France (Straib, 1935).

These results show that all the wheat varieties in differential hosts except *Triticum dicoccum tricoccum* are resistant.

Seedling resistance tests conducted at this laboratory had shown earlier that race 57 and G of *P. striiformis* are virulent mainly on barley varieties. Twenty three important barley cultures and hybrids were, therefore, screened against these known races of yellow rust as well as race 24.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Infection types produced by new race and that of race 24 of *P. striiformis* on the differential hosts (Straib 1937).

Stock Collection or Isolation	Michigan Amber	Ble Rouge D'ecose	Strubes Dickkopf	Webster	Holzapfel's Fruh	Vilmorin 23	Heins Kolben	Carsten V	Spalding Prolific	Rouge Prolific Barbu	Chinese 166	Triticun Dic. Tric.	Fongtein	Heils Franken	Petkusar Rye	Agra Local Wheat*	Agra Local Barley*
Wellington, NP. 108																	
Heils Franken(4)	0	i	i	0	i	i	0	0	i	i	0	4	4	4	i	0	4
Race 24	0	0	i	i	i	i	0	i	i	i	i	4	4	4	i to 0		

*Controls for *P. striiformis* in India.

Infection types after Gassner and Straib (1932).

Varieties EB, 1467, E. B. 1626 and strains H. W. 44, B. S. 15-60-88 and B. H. S. 37-60-37 were found to be resistant to all 3 races. Fourteen cultures, viz. B. H. Nos. 4-7-1, 4-7-33, B. H. S. Nos. 14-60-68, 37-60-39 H. W. Nos. 41, 42, 46, E. B. 410, 1370, 1719, 1738, 1179, 1880 and 1881 were resistant to races 57 and G but susceptible to race 24. E. B. 1877 was resistant to race 24 but susceptible to races 57 and G. However, E. B. 999, E. B. 1742 and E. B. 1760 were found to be susceptible to these 3 races (24, 57 and G). Resistant varieties can be used in the crossing programme

[1]Gassner, G. and W. Straib. *Arb. biol. Abt.* (*Anst. Reichanst.*), *Berl.*, **20** : 141-63 (1932).

*[2]Straib, W. *Arb. biol. Reichanst. Landu. Forstw. Ber. Dabl.*, **21** : 3, pp. 455-466. (1935.

[3]Straib, W. *Arb. Biol. Anst.* (*Reichanst.*) *Barl.*, **22** : 91-119. W. (1937).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Floury leaf spot of bean caused by *Ramularia phaseolina* **Petrak.**—H. S. SOHI, S. L. SHARMA, B. R. VERMA, and K. B. SACHDEVA, Section of Plant Pathology, Department of Agriculture, Himachal Pradesh, Solan—During the month of August and September, 1964, a leafspot of bean (*Phaseolus vulgaris* L.) was observed at Vegetable Research Station, Solan (1520 m. a. s. l.). The disease did not appear to be of importance in the beginning but subsequently became severe and caused extensive premature defoliation starting at the lowest leaves and progressing upwards.

Symptoms: The disease appeared in the form of white tufty growth of the fungus conidiophores on the lower leaf surface which gave to the leaf the appearance of having been sprinkled with coarse flour, hence the disease is named as floury leaf spot. The corresponding upper side of the leaf turned yellow, then brown and finally dried. Young leaves were not affected by the disease.

Morphology: The colourless conidiophores emerge in tufts from the stomata and intertwine in clusters around the leaf hairs, erect, more or less divergent, somewhat tapering towards the tip, simple or irregularly dichotomous, a few short, erect, single conidiophores were also found scattered over the leaf surface; conidia variable in shape and size mostly ellipsoid or ovoid, tips acute or obtuse, base papillate at the point of attachment, straight, hyaline, commonly aseptate, rarely 1 septate and measure 5.24 - 21.4 × 3.5-5.3 μ.

This disease which is caused by *Ramularia phaseoli* has so far been reported from Ecuador, Colombia, Nicaragua and East African Countries. This is the first record of this disease from India.

The authors are indebted to Mr. F. C. Deighton of the Commonwealth Mycological Institute for help in identification of the fungus.

[1]Cordana-Alvarez C. and R. L. Skiles. Plant Dis. Reptr., **42** : 778-780. (1958).

[2]Saccardo P. A. Sylloge Fungorum, **22** : 1315 (1913).

———

Bacterial blight disease of *Eleusine coracana* **Gaertn.**—S. G. DESAI, M. J. THIRUMALACHAR and M. K. PATEL, Baria Science Institute, Navsari and Hindustan Antibiotics Ltd., Pimpri, Poona.—*Eleusine coracana*, popularly known as ragi or finger millet is widely cultivated as dry-land or irrigated cereal crop in India and forms the staple food of the lower middle classes. Among a few diseases known to affect the crop are *Piricularia oryzae* Cav., *Helminthosporium nodulosum*, Berk. and Curt. and *Melanopsichium eleusinis* (Kulk.) Mundk. and Thirum. and *Sclerophthora macrospora* (Sacc.) Thirum. *et al.* A bacterial disease which is systemic in the vascular strands also occurs in several parts of India, and in the irrigated or heavy rain-fall areas, often causes considerable damage. The disease is easy to recognise, since by sectioning the infected plant parts, the bacterial ooze from the cut portions of the vascular strands is quite conspicuous. Rangaswami, Prasad and Eswaran (1961)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

noticed the disease and isolated truly a saprophte or secondary organism which was named as *Xanthomonas eleusineae* nov. sp. the incitant of the disease. Patel and Thirumalachar (1965) pointed out that *X. eleusineae* is not a *Xanthomonas* species at all, since it produces acid in salicin and the colonies are somewhat reticulate, tough, tenacious and not butyrous. This has been confirmed by study of the type culture obtained from the authors as well as those deposited in culture collections abroad. The characteristics of the organism as given by Rangaswami *et al* (1961) viz. (i) non-capsulated, (ii) non-hydrolysis of starch, (iii) reduction of nitrate, (iv) no production of ammonia and (v) little or no gas formation from lactose, differ from those of the pathogen under study.

Since the true *Xanthomonas* species inciting the bacterial blight of ragi has not been isolated and studied, except for the symptoms of the disease as given by Rangaswami *et al*, it was proposed to restudy the disease taking advantage of its occurrence in the western part of central India. In the districts of Panchmahals and Mehsana in north and Dangs district in south Gujarat, the bacterial blight of ragi occurs extensively causing considerable damage to the crop. The plants are susceptible to infection at all stages of growth. If infection takes place during early stages of growth, the plants become yellow and show premature wilting.

Infection first appears as water-soaked, translucent, linear, pale-yellow to dark greenish-brown streaks, 5 to 10 mm. long, and running parallel to the mid-rib of the lamina. The hyaline streak later develops into a broad yellowish lesion measuring 3 to 4 cms. and turns brown. When the infection is heavy, particularly in the early stages, the entire leaf turns brown and withers away.

Isolation of the disease-inciting pathogen from several localities showed that the same species was present. The type culture of *X. eleusineae* obtained from the authors showed that it was non-pathogenic and was not a *Xanthomonas* species. The morphological, cultural and biochemical characteristics of the present isolate are presented below :—

Short rods with rounded ends, usually single, occasionally in pairs, measuring $1.1\text{-}1.8 \times 0.5\text{-}0.7\ \mu$, motile by a polar flagellum, gram-negative, encapsulated, no endospore and non-acidfast. Colonies on potato-dextrose agar plates are circular with entire margin, smooth, pulvinate, butyrous and glistening yellow. Growth on potato-dextrose agar slants is abundant, filiform, convex, glistening, smooth, opaque, butyrous and lemon yellow ; medium unchanged. On nutrient agar slants, growth is moderate, filiform, convex, glistening, smooth, opaque, butyrous and lemon yellow ; medium unchanged.

Gelatin liquefied, starch hydrolysed, casein digested, tributyrin and several other fats hydrolysed, milk peptonised and litums reduced ; ammonia and hydrogen sulphide produced from peptone ; nitrates not reduced to nitrites ; indol not produced ; V. P. and M. R. tests negative ; citrates utillised but not uric acid ; tolerates 3 per cent sodium chloride ; acid without gas from arabinose, xylose, glucose, fructose,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

galactose, mannose, lactose, maltose, sucrose, cellobiose, starch, glycogen, dextrin and mannitol but not from rhamnose, inulin, salicin, sorbitol, dulcitol and inositol. Seventeen amino acids support growth as source of nitrogen while DL-serine, DL-nor-leucine and L-tyrosine failed to do so. DL-alanine, L-glutamic acid, L-proline and L-hydroxyproline support growth while sixteen other amino acids failed to support growth as source of carbon. Catalase positive ; facultative aerobe ; optimum temperature for growth 27-30°C.; thermal death point 52°C.

Inoculation experiments were carried out on both the seedlings and mature plants of *Eleusine coracana*. Typical disease symptoms were obtained, and reisolation of the same pathogen from the diseased tissue confirmed the pathogenicity of the organism. Cross inoculation experiments carried out on *Oryza sativa L.*, *Panicum miliaceum* L., *Paspalum scrobiculatum* L., *Sorghum vulgare* (L.) Pers., *Setaria italica* Beauv., *Pennisetum typhoideum* Rich. and *Zea muys* L. showed that these are not susceptible. Inoculation experiments on the closely related genus *Dactyloctenium aegyptium* Beauv. could not be carried out. The *Xanthomonas* species inciting the vascular and leaf disease in *Eleusine coracana* is a new species, and the name *X. coracanae*. Desai, Thirumalachar and Patel is proposed for its accomodation.

[1]Rangaswami, G., N. N. Prasad and K.S.S. Eswaran. Indian Phytopath. 14 : 105-7 (1961).

[2]Patel, M.K. and M.J. Thirumalachar. Curr. Sci. 34 : 436-437 (1965).

———

Trichothecium roseum link. A hyperparasite of sugarcane rust (Puccinia erianthi).—B. L. CHONA, J. C. DURGAPAL, H. C. GUGNANI* AND H. S. SOHI**, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Sreekantiah and Joshi[1] reported the development of a fluffy growth of *Trichothecium roseum* Link. on the uredo-pustules of black rust of wheat under highly humid conditions and inhibition of germination of uredospores of the rust by culture filtrate of *T. roseum*. But occurrence of *T. roseum* on uredo-pustules of sugarcane rust (*Puccinia erianthi*) is being reported here for the first time. During the routine maintenance of cultures of sugarcane rust at Delhi under glass-house conditions, in the months of August and September, 1961, it was frequently observed that when humidity in the glasshouse was very high (60-80 per cent), the uredo-pustules of sugarcane rust were covered with a pinkish-white fluffy mycelium. The fungus was isolated in pure culture and identified as *Trichothecium roseum* Link.

Since the fungus was consistently associated with the uredo-pustules of sugarcane rust, it was desired to carry out investigations on the association of these two organisms. An experiment, consisting of four treatments was set up. A concentrated spore suspension of the fungus was sprayed by an atomizer on sugarcane (Co. S. 510) seedling (i) uninjured young leaves (ii) injured young leaves (pin pricking) (iii) young leaves with well developed uredo-pustules. The fourth treatment in which

Present address: *National Institute of Communicable Diseases, Delhi.
**Plant Pathologist, Department of Agriculture, H. P., Solan.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

only distilled water was sprayed on young leaves with well-developed uredo-pustules served as a control. All were kept in humid chambers, separately, under identical conditions. After a week, it was observed that no fungal growth appeared in the treatment Nos. (i), (ii) and (iv), but a pinkish-white fluffy mycelial growth of *T. roseum* appeared on the uredo-pustules of the rust in the third treatment.

Uredospores collected from the pustule affected by *T. roseum* showed a high degree of reduction in germination. An experiment, therefore, was conducted to study the effect of the fungus on the germination of uredospores of sugarcane as well as *Erianthus* rust. In one treatment uredospores of *Puccinia erianthi* (on sugarcane) and *P. kuehnii* (on *Erianthus munja*) were separately mixed with spores of *T. roseum* in distilled water and incubated for germination. In another treatment, uredospores of both the rusts were separately kept for germination in films of distilled water containing previously germinated spores of *T. roseum*. The controls consisted of only uredospores in distilled water. Germination counts were taken after 24 hours incubation at room temperature (25-27°C).

It was observed that the percentage reduction in germination of uredospores of *P. erianthi* in the two treatments was 51.5 and 65, respectively. A similar situation was observed with *P. kuehnii* where the percentage reduction in germination of uredospores was 53.8 and 77.8, respectively, for the two treatments. It is apparent that there was a marked reduction in germination of uredospores of sugarcane and *E. munja* rusts when they were mixed with germinated spores of *T. roseum* in distilled water. This indicates that the inhibition of germination of uredospores was due to the inhibitory substance(s) secreted by the germinated spores of *T. roseum*, being present in the water film prior to the germination of uredospores of the rusts.

The effect of the culture filtrate of *T. roseum* obtained by cultivating the fungus on Potato-Dextrose-Yeast broth for 10 days was therefore studied by germinating uredospores of both the rusts in different concentrations of the filtrate. Complete inhibition of germination of uredospores of *P. erianthi* and *P. kuehnii* was observed at 15 per cent and 20 per cent and higher concentrations of the culture filtrate, respectively, but none at 0.5 per cent concentration. This property was not lost after the filtrate had been autoclaved at 15 lbs. pressure for 20 minutes thereby indicating that it is thermostable.

Grateful thanks are due to Dr. R. S. Vasudeva, formerly Head of the Division of Mycology and Plant Pathology, I. A. R. I., New Delhi and to Mr. R. L. Munjal, Systematic Mycologist, Division of Mycology and Plant Pathology for their kind interest and help.

---

[1]Sreekantiah, K. R. and L. M. Joshi, A *Trichothecium* sp. (*Cephalothecium* sp.) as a hyperparasite in *Puccinia graminis tritici* (Pers.) Erikss. and Henn. *Indian Phytopath.*, **11** (1) : 62-65 (1958).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phoma rot of tomato.—RAJENDRA K. GROVER, Department of Botany, Punjab University, Chandigarh 3, (Pb.).*—During winters of 1963-64, a disease on tomato fruits characterized by small, slightly depressed spots near the stem-end or the top portions of the fruit, which later turn brown and show erumpent pycnidia, was noticed in a severe form in the fields around Chandigarh, Punjab. Lesions on the ripe fruits were more conspicuous than on the green fruits in the field. The lesions on green fruits develop into usually circular, depressed, water soaked spots, $1\frac{1}{2}$—2 in. in diameter, which may later become leathery on the surface with prominent pycnidia on ripe fruits (Fig. 1). Leaves and stem were less frequently affected, but when diseased, the leaves showed small dark irregular spots, sometimes very numerous which turned the leaves pale and made them fall prematurely ; while on stems the lesions were dark and elongated. Jamieson[1] has given a detailed account of the disease from the United States. The present paper records the occurrence of *Phoma* rot of tomato for the first time from India.

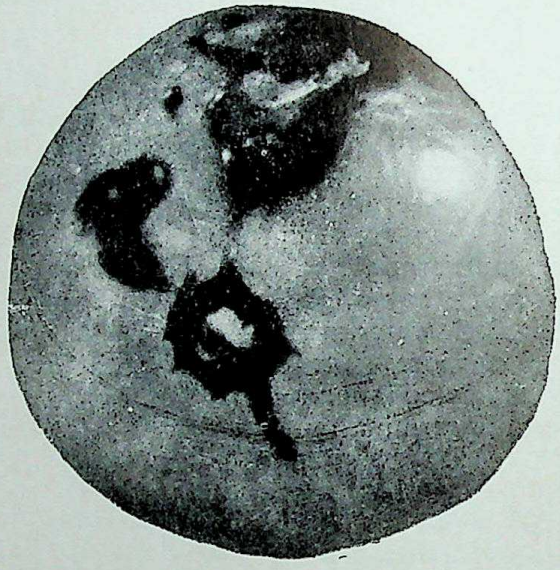


Fig. 1. Tomato fruit showing lesion of *Phoma destructiva*

Isolations from affected tomato fruits were made by the usual techniques. The organism grew luxuriantly on 2 per cent potato dextrose agar and other nutrient media tried. It formed whitish mycelia which later turned pink and became olive green to black with age. The mycelium is septate, branched and hyaline when young but turns dark when old, and possesses abundant oil bodies in it. Abundant minute pin-head sized pycnidia were observed in cultures when incubated for 5-7 days at 28±1°C. Exposure of cultures to light greatly favoured the production of pycnidia. Pycnidia are dark coloured, sub-globose to globose in shape, slightly papillate with 1 or 2 or more ostioles, measuring 30-280 μ in diameter. Abundant pycnospores are exuded

*Present address : Dept. of Botany and Plant Pathology, Punjab Agricultural University, Hissar.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

out of pycnidia in cirri under moist conditions. The pycnospores are flesh coloured *en masse*, hyaline, continuous, sub-cylindrical to sub-globose, measuring 1.4—2.8 × 3.5-8.4 μ. The pycnospores germinate readily in water in 4-5 hours. On the basis of above morphological characters the organism is identified as *Phoma destructiva* Plowr.

Pathogenic nature of the organism was confirmed by inoculating healthy tomato fruits. Infections on wounded tomatoes was rapid and more successful than in the unwounded fruits. Progress of disease was more rapid in ripe than in green fruits under moderate temperatures and high humidity. Pycnidia developed on diseased tissues within 6-7 days after inoculation. Besides tomatoes, the fruits of brinjal (*Solanum melongena* L.), datura (*Datura stramonium* L.), chilli (*Capsicum annum* L.) and *Withania somnifera* L. could be readily infected under artificial conditions. No infection occurred on potato tubers.

Thanks are due to Prof. P. N. Mehra for facilitating these investigations.

[1]Jamieson, C. O. *J. agric. Res.*, 4 : 1-116, (1915).

———

A note on the occurrence of perfect stage of bottle gourd powdery mildew in Mysore—V. RAJENDRAN, Agricultural Research Institute, Hebbal (Mysore) —The occurrence of powdery mildew on cucurbits is very common in Bangalore but the perfect stage of the pathogen is a rarity particularly in South India. In Mysore there has been so far no report of the occurrence of the perithecia of *Erysiphe cichoracearum* DC.

While studying the powdery mildews of various hosts in Mysore State, cleistothecia of *E. cichoracearum* were observed on bottle gourd plants (*Lagenaria vulgaris* Ser.). The plants were grown in 6″ pots in cages under shade. The plants were heavily diseased due to natural infection. The conditions prevalent were cool, humid and complete shade without direct sun light.

The perithecia were observed as dark dots interspersed in the mildew patches present on both the dorsal and ventral surfaces of the infected leaves. The perithecia varied in size from 51 to 123 μ in diameter. Some of them were observed to be in the very early stages of development. The appendages were variable, long or short, coloured or hyaline, numerous, densely interwoven, branched, septate, *12 to 594* μ, sometimes very few and short or even rudimentary. When the apparently mature cleistothecia were crushed under the coverslip, asci-like structures were observed. No ascospores were formed. A similar observation has been made by Stone (1961)[1] in the case of *E. cichoracearum* on *Sonchus asper* Gaertn.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Figs. 1–2. Cleistothecia on the bottle gourd leaf. Cleistothecia (a) Appendages. (b) Ascus.

The formation of perithecia of the powdery mildews and particularly their maturation and development of ascospores is influenced by various factors such as the physiology of the host as well as the pathogen, and the environment.

Even the immature cleistothecia of *E. cichoracearum* on bottle gourd failed to appear subsequently in 1964. Further work is in progress.

ACKNOWLEDGEMENT : I wish to thank Dr. H. C. Govindu, Plant Pathologist, Agricultural Research Institute, Hebbal, for guidance.

[1]Stone, Olwen M. *Ann. appl. Biol.* **50** : 203 - 210 (1962).

———

A note on mango fruit-rot in storage—DHARAM VIR AND RAJESHWARI SHARMA, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi—During investigations on the storage-rot of mango fruits, *Sclerotium rolfsii* was isolated from variety *Dusheri* collected from local fruit market. The disease was characterised by the formation of deep grey lesions scattered all round the surface of fruits. The lesions ranged from 1—2 cm in size and had irregular boundary. The market value of this important

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

fruit is considerably reduced because of the intense discoloration of fruits. The pathogenic nature of the fungus was confirmed by inoculating healthy mango fruits of the same variety with this fungus. This was done by giving an injury to the fruits as also by inoculating on the intact fruit surface. The rotting developed only in those fruits where an artificial injury had been inflicted. The symptoms of the disease appeared after 4-5 days and there was complete rotting of the fruits in 8—10 days (Fig. 1). There is no mention of mango fruit-rot caused by this fungus so far and this appears to be the first record of its type in India.

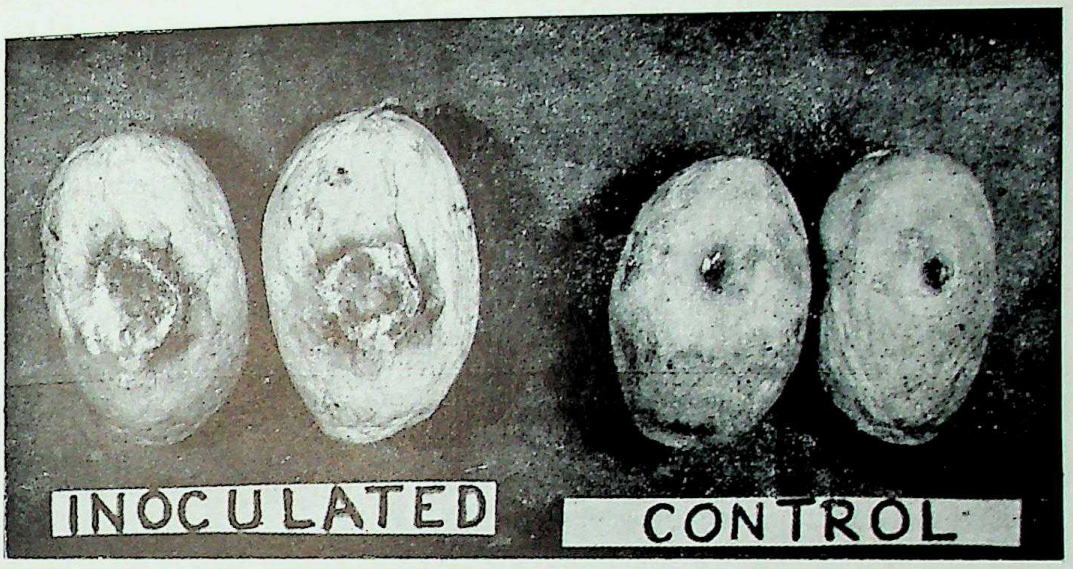


Fig. 1. The rotting of mango fruits after inoculation with *Sclerotium rolfsii*.

In order to study varietal resistance if any, inoculations were made on five fruits of each of the varieties of *Langra*, *Sehroli* and a local variety. Fruits of all these varieties developed rotting, showing thereby that they were susceptible to this pathogen. Further studies with regard to the factors affecting the disease development in transit and storage as also evolution of suitable control measures, are in progress.

ACKNOWLEDGEMENT : Thanks are due to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology for his keen interest in this work.

———

Meloidogyne sp. in rhizomes of Ginger (Zinziber officinale Rosc).—S. N. KULKARNI AND A. C. JAIN, Plant Pathology Department. J. N. Krishi Vishwa Vidyalaya Jabalpur.—During the course of investigation into rhizome rot of ginger, presence of root galls incited by nematodes were noted on roots. Examination of rhizomes by cutting them with a sharp knife revealed the presence of pearly white females embedded in the tissue. Root-knot of ginger caused by *Meloidogyne* sp. has been reported in India from Kerala[1]. However, the presence of females of *Meloidogyne* in the rhizomes has not so far been recorded.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The rhizomes harbouring females cannot be differentiated from healthy ones by only external examination. These females can only be detected by cutting such apparently healthy rhizomes. About 7 per cent of rhizomes collected from Chhindwara, were found to be harbouring nematodes. The females and egg-masses are deeply embedded in the tissue, sometimes as deep as 3 mm. from the surface of rhizomes. In earlier stages the females are pearly white and the surrounding tissue is not discoloured, but later the host cells in contact with egg-mass turn brown and the position of female can be easily located by a small brown spot.

The presence of females and their egg-masses deeply embedded in the rhizomes indicate that these infected rhizomes are a potential source of dissemination and perpetuation of this pathogen. An experiment was laid out in 10" earthen pots by using steam sterilised sandy loam soil to verify this observation. The heavily infected rhizomes from previous season were added to these pots. Out of the 20 pots, 10 were fumigated with 0.5 ml. of nemagon/pot. The third set of 10 pots with sterilised soil received neither nematode inoculum nor fumigation, being check. The soil in pots was kept moist. After 30 days of fumigation (period sufficient to remove phytotoxic effect of nemagon) 3 mature rhizomes of ginger from nematode free stock, were planted in each pot. At maturity of the crop, the ginger plants along with rhizomes and roots were removed and examined for nematode infection. The results of the examination are given below.

Nematode infection of ginger plants as affected by different treatments.

Treatment	*Observation*
1. Sterilised soil alone	There was no nematode infection either of rhizomes or roots.
2. Sterilised soil with infected pieces of rhizomes.	Nematodes were invariably found in each of the newly formed rhizomes and there was abundant gall formation on the roots.
3. Sterilised soil fumigated after adding infected pieces of rhizomes	There was no nematode infection either of rhizomes or roots.

The results show that the infected ginger rhizomes used for planting serve as a source of dissemination and perpetuation of nematodes. Therefore use of disease free seed will be one of the important factors in controlling this nematode.

It was also shown by the above experiment that this nematode can be checked by fumigation and therefore where the soil is infected, fumigation of the same will also have to be done.

[1]A. M. Nadkal, *Curr. Sci.* 32 : 360-61 (1963).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Ear rot of maize caused by Cephalosporium acremonium corda, a new record for India.—K. S. DHANRAJ AND S. B. MATHUR, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Maize ear rots continue to be of importance wherever maize is grown and there are various organisms associated with these rots. (1, 2) So far little information is available on the occurrence of ear rots in India. Ear rot and seed-infection caused by *Cephalosporium acremonium* Corda has been reported by Butler (3) from Australia and Koehler (4) from the U. S. A. According to Reddy and Holbert (5) this organism also causes "black bundle" disease of maize. Stalk rot of maize observed in Rajasthan and Mysore during 1963-64 was found to be caused by *C. acremonium* (RF Report) (6).

During a disease survey of maize made in August 1964, an ear rot caused by *Cephalosporium acremonium* was observed at Siliguri and Kalimpong (West Bengal) and Gangtok (Sikkim), on local varieties and hybrid maize. Disease incidence was greater on the hybrid, Ganga Hybrid Makka 101 and on the variety, Amarillo-de-Cuba (Ade Cuba). There was not external symptoms apparent on most of the diseased plants. When husks were removed, however, white fluffy mycelium was present on the entire surface of some of the ears and was particularly dense in the grooves between the kernel rows. Small white streaks occurred on most of the kernels of the infected ears which has been reported as characteristic of *C. acremonium* infection (Koehler 1942). This ear rot was approximately 30 per cent prevalent and general blight occurred on a few plants and was accompained by a reddening or purpling of leaves and stalks. Multiple ear production from a single node was common in infected plants of Ade Cuba variety. None of the healthy plants of the same variety in the field showed this phenomenon.

The streaks in the infected kernels were more pronounced on the lower half of the kernel surface. Most of the infected seed looked normal in shape and size. Only a few infected grains were shrivelled. In shrivelled grain, the germ area became purplish or sometimes pink in colour. Microscopic examination of the diseased seed revealed the presence of the spores of *C. acremonium*. 90 per cent of the streaked seeds yielded white fluffy mycelium of the pathogen after four days incubation, which turned pink within eleven days due to profuse spore production.

Even though infection by the fungus does not inhibit the germination or affect the vigour of the sprouts under laboratory conditions, the quality of the grains is affected due to the presence of streaks and discolouration of kernels. This is the first record of the cob rot due to *C. acremonium* in India, and has particular significance as the disease is associated with hybrids which were examined in the area. This disease was not found in the rabi crop at Godhra in Gujarat State, Hyderabad and Kurnool in Andhra Pradesh.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENT : The authors wish to thank sincerely Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology for providing necessary facilities for work and for critically going through the manuscript and giving helpful suggestions. Our grateful thanks are also due to Mr. J. K. Thapa, Assistant Maize Breeder, Gangtok, Sikkim for all the help rendered during the survey.

---

[1]Dhanraj, K. S. and D. Suryanarayana. *Proceedings Annual Conference of the Coordinated Maize Improvement Scheme* (1965). (Unpublished).

[2]Koehler, B. Corn Ear Rot in Illinois. *Ill. Agric. Exp. Sta. Bul.* 639 1959).

[3]Butler, F. C. *Agric. Gaz. N. S. W.*, **48** : 144-155 (1947).

[4]Koehler, B. *J. agric. Res.*, **54** : 421-442 (1942).

[5]Reddy, C. S. and J. R. Holbert. *J. agric. Res.*, **27** : 177-205 (1924).

[6]Rockefeller Agricultural Sciences Annual Report 63-64, p. 197.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological News

1. CONGRATULATIONS TO GENETICIST M. S. Swaminathan, Head, Division of Botany, Indian Agricultural Research Institute on his being honoured with Birbal Sahni Medal for the year 1965, by the Indian Botanical Society.

2. PHYTOPATHOLOGIST R. S. Mathur of Uttar Pradesh Government, joined the Department of Agriculture in Iraq as a Technical expert for investigations on diseases of date palm.

3. I. P. S. CONGRATULATES PHYTOPATHOLOGIST H. C. Govindu on his being appointed as Professor of Plant Pathology, Agricultural University, Bangalore.

4. CONGRATULATIONS TO PHYTOPATHOLOGIST S. P. Raychaudhuri, Head, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, on his election as Fellow of the National Institute of Scie nces of India.

5. Dean G. Rangaswamy, Agricultural University, Bangalore has left on a study tour of continent and U. S. A. to study the systems of agricultural education in these countries.

6. I. P. S. extends warm greetings and felicitations to the Phytopathological Society of Netherlands, Wageningen, Netherlands on its 75th Anniversary celebrations in April, 1966.

7. VIROLOGIST Robley C. Williams, Associate Director, Virus Research Laboratory, University of California, Berkeley, U. S. A., addressed the I. P. S. on January 2, 1966, during the Annual General Meeting held at Chandigarh.

8. PHYTOPATHOLOGIST R. L. Mathur of Rajasthan (Jaipur) has returned to India after one year advanced training in virology in the Department of Agriculture, Vancouver, Canada.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Indian Phytopathology

VOLUME XVIII, 1965

Consisting of ix+395 Pages

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

No. 1 JANUARY-MARCH

PAGES

Microbiological complex of the Phyllosphere and disease control—*S. Sinha* 1

Carbon requirements of Colletotrichum inamdarii—*S. K. Hasija* 21

On two unrecorded races and a new biotype of Puccinia recondita—*M. M. Payak* 26

A new rust on Schizandra grandiflora—*Hardev Singh and S. Jalan* 29

Seed microflora I. Seed-borne fungi of ragi (Eleusine coracana Gaertn.) their distribution and control—*J. S. Grewal and Mahendra Pal* 33

Leaf blight and dry stalk rot of strawberry caused by Rhizoctonia bataticola—*V. C. Lele and H. C. Phatak* ... 38

Studies on leaf blight of Inga dulcis—*A. K. Roy* ... 43

Cultivation of some plant viruses in callus tissue cultures—*S. P. Raychaudhuri and M. D. Mishra* 50

The genus Pringsheimia in India—*S. T. Tilak and Ramchandra Rao* 54

Studies on cape gooseberry mosaic disease—*S. P. Capoor and D. C. Sharma* 58

Rusts connected with barberry in Central Nepal—*D. P. Misra* 66

Some new records of the order Mucorales from India—*A. K. Sarbhoy* 71

Phytopathological Notes

Inhibition of sporulation by light in Alternaria triticina Prasada and Prabhu—*A. S. Prabhu and R. Prasada* ... 81

To evaluate methods of applying 1, 2-di-bromo-3 Chloropropane for the control of nematode root-galls—*R. B. S. Birat* 82

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

A virulent biotype of physiologic race 107 of brown rust of wheat : Puccinia recondita Rob ex. Desm—*R. Prasada, S. K. Sharma and Madan Mohan* 84

A new leaf-spot disease of singhara (Trapa bispinosa) caused by Bipolaris tetramera—*G. P. Singh and S. Lal* 85

"Purple leaf" disease of beet—*S. P. Capoor and D. C. Sharma* 88

Two powdery mildews from Sikkim—*J. N. Kapoor* ... 90

An undescribed Myxomycete from the coffee estates of Mysore—*V. Agnihothrudu* 92

Relative efficacy of some new fungicides for the control of hill bunt of wheat—*J. S. Grewal, P. C. Joshi, K. D. Pathak and S. B. Mathur* 94

Deodar fruit extract—an inhibitor of chilli mosaic virus—*S. P. Raychaudhuri and K. C. Chadha* 94A

Reviews 97

Eighteenth Annual Report of the Indian Phytopathological Society for the year 1964. 99

Manuscripts for Indian Phytopathology 103

Phytopathological News 106

No. 2 APRIL-JUNE

Raghunath Prasad Asthana, 1906—1965 i

Study of heteroecious life-cycles of rusts in India—*M. J. Narasimhan* 107

Nutritional requirement of Gloeosporium musaram—*A. P. Misra and D. P. Thakur* 116

Seed microflora II. Seed-borne fungi of Setaria italica, their distribution and control—*J. S. Grewal and Mahendra Pal* ... 123

Tests with different varieties of sesamum for resistance to Phytophthora blight of sesamum—*P. D. Gemawat and N. Prasad* 128

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Mutation of Colletotrichum falcatum the causal organism of red rot of sugarcane V. Pathogenicity of biochemical mutants—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* 133

Studies of mosaic disease of Eleusine in the Deccan—*D. G. Rao, P. M. Varma and S. P. Capoor* 139

Studies on the uptake and translocation of labelled fungicides in plants—II. TRANSLOCATION ON FOLIAR APPLIED S^{35} IN POTATO—*Dharam Vir and B. S. Bajaj* ... 151

Utilization of amino acids as sole source of carbon and nitrogen by some Xanthomonads—*W. V. Kotasthane, A. C. Padhya and M. K. Patel* 154

Factors affecting conidial production of Helminthosporium oryzae—*S. B. Chattopadhyay and C. Das Gupta* ... 160

Studies on the pathogenicity and host-range of three species of root-knot nematodes—*A. Chidambaranathan and G. Rangaswami* 168

Evaluation of host specificity character in the differentiation of Xanthomonas species—*M. K. Patel, M. C. Srinivasan and M. J. Thirumalachar* 174

Toxicity, physical properties and biological spectrum of certain copper and organic fungicides—*A. P. Misra and T. B. Singh* 181

Studies on sugarcane rust in India—*M. L. Sahni and B. L. Chona* 191

Phytopathological Notes

Serological relationship of sunnhemp mosaic virus with some other mosaic viruses commonly occuring in Delhi—*G. P. S. Anand and H. S. Sahambi* 204

Preliminary biochemical observations of leaf spot disease of groundnut—*V. Ravindra Nath, L. G. Kulkarni and B. Appaji Rao* 206

Some important observations on the staining of bacterial flagella with modified Bailey's method—*Y. P. Rao and V. K. Mathur* 208

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Notes on powdery mildews from India-III.—*G. Lall and J. N. Gupta* 210

Natural occurrence of specialised forms of Puccinia graminis and P. striiformis on Lolium perenne.—*D. P. Misra, S. T. Ahmad and Sheodhan Singh* 214

Relative efficacy of fungicides for the control of Cladosporium variabile causing leaf spot disease of spinach (*Spinacia oleracea*)—*R. L. Mathur and S. P. Sehgal* ... 215

A Fusarium sp. with cellulolytic and lignolytic properties from manure pit—*G. Rangaswami and P. Rajasekaran* ... 217

Anthracnose of Celosia argentea var. cristata in Delhi—*R. L. Munjal and J. N. Gupta* 218

Occurrence of Tristeza virus in citrus in Northern India—*T. K. Nariani, H. S. Sahambi and B. L. Chona* ... 220

Reviews 221

Phytopathological News 224

No. 3 JULY-SEPTEMBER

Chemical treatment of seed and nursery bed to control damping-off of cabbage—*J. S. Grewal and R. P. Singh* ... 225

Performance of oat varieties against Puccinia coronata avenae and Puccinia graminis avenae—*D. P. Misra, Sheodhan Singh and S. T. Ahmad* 229

A mosaic disease of tori (Luffa acutangula)—*D. K. Mitra and T. K. Nariani* 233

Hainesia lythri on strawberry—A new record for India—*H. C. Phatak and M. M. Payak* 237

Collar-rot of loquat by Sclerotium sp. and its control—*I. N. Tandon* 240

Assay of Bordeaux Mixture as soil fungicide—*O. P. Sharma and A. C. Jain* 246

The effect of stem rust on the uptake and incorporation of P^{32} by wheat seedlings—*K. L. Mukherjee and Michael Shaw* 250

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Mutation in Colletotrichum falcatum, the causal organism of red rot of sugarcane-VI. Induced biochemical mutants as test organisms for bioassay of P-aminobenzoic acid and Biotin—*B. S. Bajaj, M. S. Chatrath and R. S. Vasudeva* 253

A pathogenic Cunninghamella on pumkin and its control—*Rajendra K. Grover* 257

Studies on Puccinia hordei I. Physiologic specialization and screening of varieties—*L. M. Joshi, R. Prasada and L. B. Goel* 267

Effect of hexose phosphates and ultraviolet irradiation on perithecial development in Glomerella tucumanensis—*A. K. Dutta, B. L. Chona and B. S. Bajaj* 274

Extractability of nucleic acids from the callus tissues of Phylloxera gall and grape stem—*H. C. Arya* ... 282

Additional viral diseases of temperate fruits in Simla Hills—*B. B. Nagaich and K. S. Vashisth* 288

Studies on the stimulation of conidial germination in Colletotrichum falcatum—*Pritam Singh* 291

Effect of maleic hydrazide on rusts of wheat and barley—*L. M. Joshi* 303

Phytopathological Notes

On the occurrence of Botryosporium palmicolum Speg. in India—*P. N. Mathur and G. Singh* 309

A mosaic disease of runner bean—*K. S. Vashisth and B. B. Nagaich* 311

Helminthosporium rostratum on maize in India—*T. P. Bhowmik and R. Prasada* 312

A mosaic disease of tree tomato (Cyphomandra betacea Sendt.)—*R. D. Joshi and K. S. Bhargava* 313

A new Phyllosticta species on sorghum in India—*D. Suryanarayana and Mahendra Pal* 314

Morus indica—An additional host of fig mosaic—*K. S. Vashisth and B. B. Nagaich* 315

Tobacco broken ring spot virus from India—*K. S. M. Sastry* ... 316

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Observations on a mosaic disease of sunflower—*A. N. Battu and H. C. Phatak* 317

Further observations and studies on pigeon pea sterility—*M. L. Seth* 317

A note on aphid transmission of mosaic disease of mulberry—*S. N. Chatterjee and S. P. Raychaudhuri* ... 319

Abstracts of papers presented at the Indian Phytopathological Society meeting held at Calcutta on Dec. 30, 1964 321

Phytopathological News 326

No. 4 OCTOBER-DECEMBER

Additions to the fungus flora of Assam-I—*A. K. Roy* ... 327

Metabolites of Fusarium oxysporum F. cumini in relation to cumin wilt—*B. L. Mathur and R. L. Mathur* ... 335

Central Himalayan fungi-II—*S. K. Bose aud Emil Muller* ... 340

Studies on Puccinia hordei II—Formation and germination of teleutospores—*L. M. Joshi* 356

The genus Macrolepiota in India—*R. N. Ghosh and N. C. Pathak* 360

Mutation in race 107 of brown rust of wheat Puccinia recondita—*S. K. Sharma and D. P. Misra* 363

Mycostatin for prevention of anthracnose of bananas in storage—*Andal Ananthanarayanan and K. Seshadri* ... 367

Further studies on chirke disease of large cardamom (Amomum subulatum Roxb.)—*S. P. Raychaudhuri and B. Ganguly* 373

Phytopathological Notes

Effect of fungicides on the viability of Brassica seeds—*S. B. Mathur* 378

Effect of zinc and amino acids on the growth of Fusarium oxysporum f. cumini Prasad and Patel—*B. L. Mathur and H. C. Sankhla* 379

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PAGES

Utilization of growth regulators by Fusarium oxysporum f. cumini Prasad and Patel—*H. C. Sankhla, B. L. Mathur and R. L. Mathur* 381

A virulent race of Puccinia striiformis West. on barley and sources for its resistance—*Sheodhan Singh, S. T. Ahmad and D. P. Misra* 382

Floury leaf spot of bean caused by Ramularia phaseolina Petrak.—*H. S. Sohi, S. L. Sharma, B. R. Verma and K. B. Sachdeva* 384

Bacterial blight disease of Eleusine coracana Gaertn—*S. G. Desai, M. J. Thirumalachar and M. K. Patel* ... 384

Trichothecium roseum link. A hyperparasite of sugarcane rust (Puccinia erianthi)—*B. L. Chona, J. C. Durgapal, H. C. Gugnani and H. S. Sohi* 386

Phoma rot of tomato—*Rajendra K. Grover* 388

A note on the occurrence of perfect stage of bottle gourd powdery mildew in Mysore—*V. Rajendran* 389

A note on mango fruit-rot in storage—*Dharam Vir and Rajeshwari Sharma* 390

Meloidogyne sp. in rhizomes of ginger (Zinziber officinale Rosc)—*S. N. Kulkarni and A. C. Jain* 391

Ear rot of maize caused by Cephalosporium acremonium Corda, a new record for India—*K. S. Dhanraj and S. B. Mathur* 393

Phytopathological News 395

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDEX TO AUTHORS

Agnihothurdu, V. ... 92
Ahmed, S. T. ... 214, 229, 382
Anand, G. P. S. ... 204
Anantharayananan A. 367
Appaji Rao, B. ... 206
Apparao, A. ... 321, 324
Arya, H. C. ... 282, 321
Bajaj, B. S. 133, 151, 253, 274
Battu, A. N. ... 317
Bhargava, K. S. ... 313
Bhowmik, T. P. ... 312
Birat, R. B. S. ... 82, 322
Bose, S. K. ... 340
Capoor, S. P. ... 58, 88, 139
Chadha, K. C. ... 94A
Chatrath, M. S. ... 133, 253
Chatterjee, S. N. ... 319
Chattopadhyay, S. B. 160
Chidambaranathan, A. 168
Chona, B. L. 191, 220, 274, 386
Das Gupta, C. ... 160
Desai, S. G. ... 384
Dhanraj, K. S. ... 393
Dharam Vir ... 151, 390
Durgapal, J. C. ... 386
Dutta, A. K. ... 274
Ganguly, B. ... 373
Gemawat, P. D. ... 128
Ghosh, R. N. ... 360
Goel, L. B. ... 267
Grewal, J. S. ... 33,94,123,225
Grover, R. K. ... 257, 323, 388
Gugnani, H. C. ... 386
Gupta, J. N. ... 210, 218
Gupta, S. C. ... 323
Hasija, S. K. ... 21
Jain, A. C. ... 246, 391
Jalan, S. ... 29
Joshi, L. M. ... 257, 303, 356
Joshi P. C. ... 94
Joshi, R. D. ... 313
Kapoor, J. N. ... 90
Khan, A. M. ... 324
Kotasthane, W. V. 154
Kulkarni, L. G. ... 206
Kulkarni, S. N. ... 391
Kumar, M. ... 323
Kumar, S. ... 323
Lall, G. ... 210
Lal, S. ... 85
Lele, V. C. ... 38
Mahadevan, A. ... 323
Mathur, B. L. ... 335, 379, 381
Mathur, P. N. ... 309
Mathur, R. L. ... 215, 335, 381
Mathur, S. B. ... 94, 378
Mathur Suresh, B. ... 393
Mathur, V. K. ... 208
Mishra, M. D. ... 50
Misra, A. P. ... 116, 181
Misra, D. P. ... 66, 214, 229, 363, 382
Mitra, D. K. ... 233
Mohan, M. ... 84
Mukherjee, K. L. ... 250
Müller, E. ... 340
Munjal, R. L. ... 218
Nagaich, B. B. ... 288, 311, 315
Narasimhan, M. J. ... 107
Nariani, T. K. ... 220, 233
Padhya, A. C. ... 154
Pal, M. ... 33, 123, 314
Patel, M. K. ... 154, 174, 384
Pathak, K. D. ... 94
Pathak, N. C. ... 360

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Payak, M. M. 26, 98, 223, 237
Phatak, H. C. ... 38, 237, 317
Prabhu, A. S. ... 81
Prakash, D. ... 324
Prasada, R. .. 81, 84, 267, 312
Rajasekaran, P. ... 217
Rajenderan, V. ... 389
Ramakrishna V. ... 324
Rangaswami, G. ... 168, 217
Rao, D. G. ... 139
Rao, R. ... 54
Rao, Y. P. ... 208
Ravindra Nath, V. 206
Raychaudhhuri, S. P. ... 50, 94A 97, 98, 221, 223, 319, 373
Roy, A. K. ... 43, 327
Sachdeva, K. B. ... 384
Sahambi, H. S. ... 204. 220
Sahni, M. L. ... 191
Saksena, H. K. ... 325
Sankhla, H. C. ... 379, 381
Sarbhoy, A. K. ... 71
Sastry, K. S. M. ... 316
Sehgal, S. P. ... 215
Seshadri, K. ... 367
Seth, M. L. ... 317
Sharma, D. C. ... 58, 88
Sharma, O. P. ... 246
Sharma, Rajeshwari 390
Sharma, S. K. ... 84, 363
Sharma, S. L. ... 384
Shaw, M. ... 250
Singh, G. ... 309
Singh, G. P. ... 85, 325
Singh, H. ... 29
Singh, P. ... 291
Singh, R. P. ... 225
Singh, Sheodhan ... 214, 229, 382
Singh T. B. ... 181
Sinha, S. 1
Sohi, H. S. ... 384, 386
Srinivasan, M. C. ... 174
Suryanarayana, D. 314
Tandon, I. N. ... 240
Thakur, D. P. ... 116
Thirumalachar, M. J. 174, 384
Tilak, S. T. ... 54
Varma, P. M. ... 139
Vasudeva, R. S. ... 133, 253
Vashisth, K. S. ... 288, 311, 315
Verma, B. R. ... 384

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

PAGES

Flouty leaf spot of bean caused by Ramularia phaseolina Petrak.—*H. S. Sohi, S. L. Sharma, B. R. Verma and K. B. Sachdeva* 384

Bacterial blight disease of Eleusine coracana Gaertn—*S. G. Desai, M. J. Thirumalachar and M. K. Patel* ... 384

Trichothecium roseum link. A hyperparasite of sugarcane rust (Puccinia erianthi)—*B. L. Chona, J. C. Durgapal, H. C. Gugnani and H. S. Sohi* 386

Phoma rot of tomato—*Rajendra K. Grover* 388

A note on the occurrence of perfect stage of bottle gourd powdery mildew in Mysore—*V. Rajendran* 389

A note on mango fruit-rot in storage—*Dharam Vir and Rajeshwari Sharma* 390

Meloidogyne sp. in rhizomes of ginger (Zinziber officinale Rosc)—*S. N. Kulkarni and A. C. Jain* 391

Ear rot of maize caused by Cephalosporium acremonium Corda, a new record for India—*K. S. Dhanraj and S. B. Mathur* 393

Phytopathological News 395

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00
Cost of Foreign Art Paper with Printing										
2 Pages	3.12	4.25	5.35	6.50	9.75	13.00	16.00	19.00	26.00	32.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1965

President ...	... M. J. THIRUMALACHAR
Immediate Past President ...	... S. SINHA
Vice-President ...	... K. S. THIND
Secretary-Treasurer ...	... S. P. RAYCHAUDHURI (1965-67)
Joint Secretary... ...	... M. M. PAYAK (1965-67)

COUNCILLORS

Northern Zone ...	... T. K. NARIANI
Mid-Eastern Zone ...	... B. M. GUPTA
Eastern Zone ...	... S. N. BANNERJEE
Central Zone ...	... B. L. MATHUR
Western Zone ...	... S. P. CAPOOR
Southern Zone ...	... G. RANGASWAMI

EDITORIAL BOARD FOR 1965—67

R. PRASADA

Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU
L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 30/- (£ 2-10-0 or $ 6.50), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

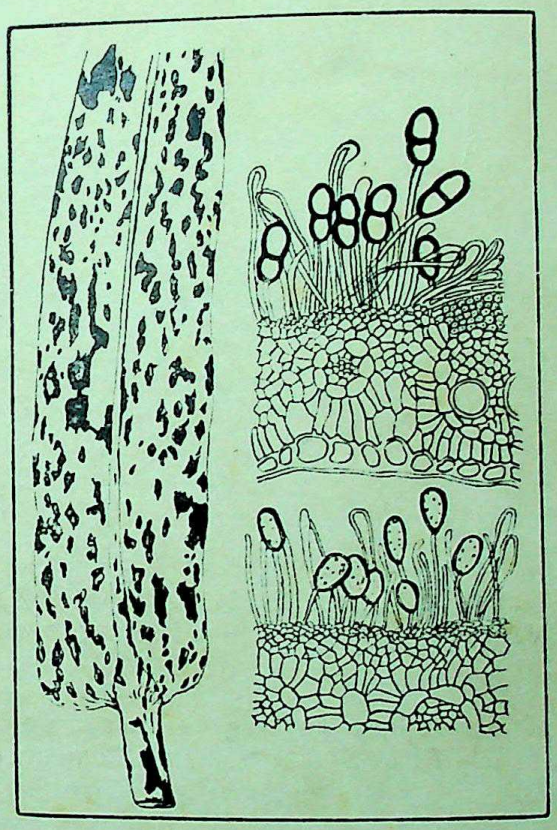
Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHOLOGY

Vol. XIX March 1966 No. 1



Published for

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

INDIAN AGRICULTURAL RESEARCH INSTITUTE

NEW DELHI-12, (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

Honorary Fellows elected in 1966 1

PRESIDENTIAL ADDRESS : Conspectus of our knowledge in the genera of Ustilaginales - *M. J. Thirumalachar* ... 3

Studies on the viability of sclerotia of Sclerotium oryzae Catt, the incitant of stem rot of paddy in Bihar—*A. P. Misra, Abu Mohammad and P. K. Das* 14

A mosaic disease of petunia—*S. C. Misra and V. V. Chenulu* 19

Nematocidal properties of Anagallis arvensis L. extracts—*Y. L. Nene and P. N. Thapliyal* 26

Studies on the effect of certain nutrients on the conidial germination in Colletotrichum falcatum—*Pritam Singh* 30

The effect of calcium, magnesium, and sulphur nutrition of guar plants on local lesion formation by sunnhemp mosaic virus—*K. S. M. Sastry* 38

Occurrence of physiologic races of wheat and barley rusts in India during 1957-62—*R. Prasada, V. C. Lele, L. M. Joshi, Sheodhan Singh, M. M. Payak, D. P. Misra, G. Swarup, L. B. Goel, G. Krishna and S. K. Sharma* 45

Changes in the free amino acids and C : N ratios of banana leaves infected with fungal pathogens—*G. Rangaswami and S. Natarajan* 59

On the taxonomy of the fungus causing anthracnose of Dioscorea alata L.—*R. D. Singh, N. Prasad and R. L. Mathur* 65

Helminthosporium disease of rice VIII. Breeding resistant varieties : selection of resistant varieties of early duration from genetic stock—*S. Y. Padmanabhan, D. Ganguly and G. H. Chandwani* 72

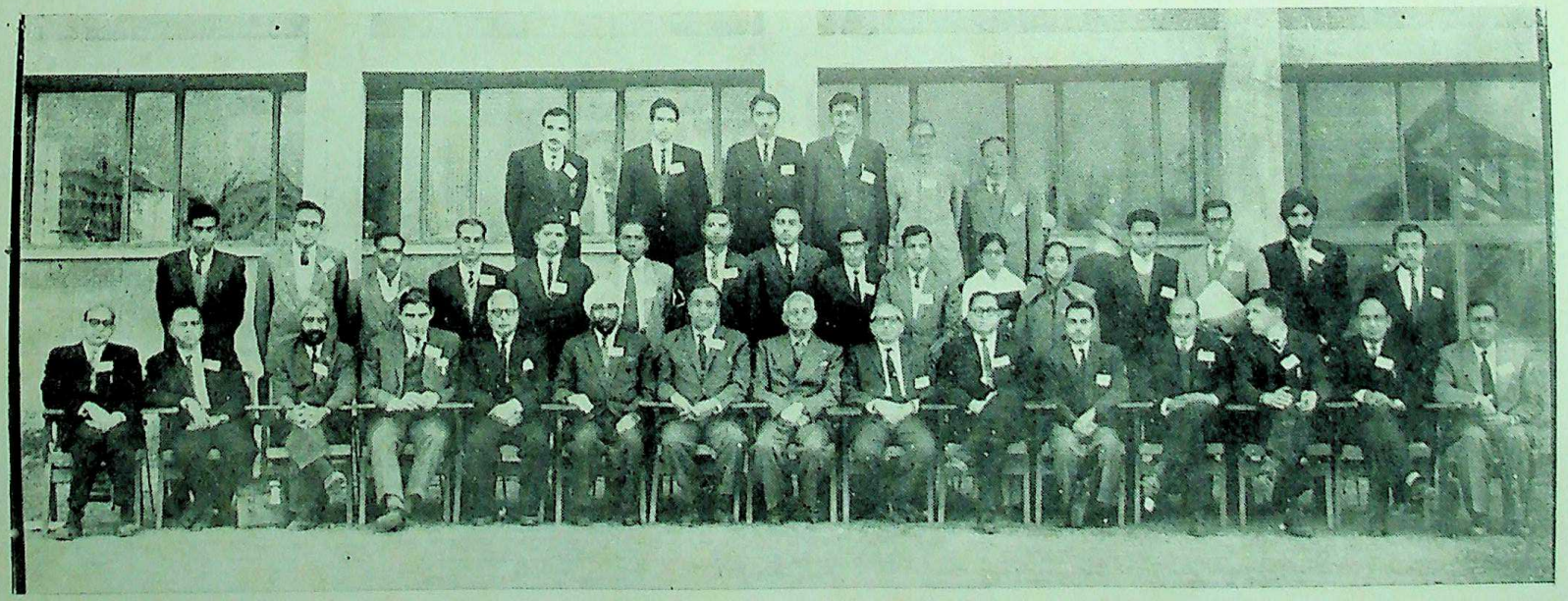
Spore dispersal in Piricularia oryzae Cav.—*A. Ramalingam* ... 76

White root-rot disease of apple in Himachal Pradesh—*R. K. Agarwala and V. C. Sharma* 82

(*Contd. on 3rd cover*)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

INDIAN PHYTOPATHOLOGICAL SOCIETY XVIII ANNUAL MEETING 1966 CHANDIGARH



Sitting L. to R. : Dr. H. C. Arya, Dr. H. C. Govindu, Dr. K. S. Bedi, Dr. E. W. Sprague, Dr. R. Prasada, Dr. K. S. Thind, Dr. M. J. Thirumalachar, Dr. K. D. Bagchee, Dr. P. N. Mehra, Dr. S. P. Raychaudhuri, Dr. M. M. Payak Dr. G. Rangaswami, Dr. B. L. Renfro, Dr. B. S. Bajaj, Dr. T. Sreeramulu.

Standing 1st Row : L. to R. Dr. M. M. Tiwari, Sh. M. D. Misra, Sh. S. B. Puranik, Dr. P. D. Varadarajan, Dr. A. Appa Rao, Dr. N. V. Sundaram, Sh. S. K. Sinha, Dr. J. N. Chand, Sh. P. D. Kharbanda, Sh. Krishan Kumar, Miss. Kanti Jain, Mrs. E. K. Rohini, Dr. Y. L. Nene, Dr. M. R. Sharma, Sh. H. S. Sohi, Dr. S. K. Batra.

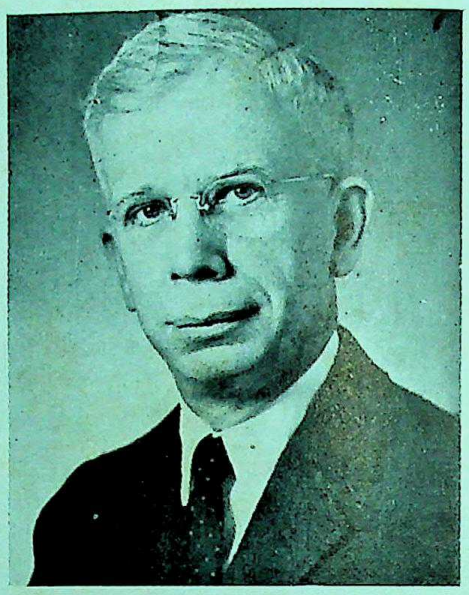
Standing 2nd Row : L. to R. Sh. S. P. S. Beniwal, Sh. A. K. Gautam, Sh. M. S. Rane; Sh. J. P. Singh, Sh. G. Lall, Dr. T. Bhowmick.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Honorary Fellows of The Indian Phytopathological Society Elected in 1966



J. G. Horsfall

Teikichi Fukushi

J. F. Dastur

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

J. G. Horsfall

Born on January 9, 1905, at Mountain Grove Missouri (U. S. A), B. Sc. in Entomology from University of Arkansas in 1925 and Ph. D. in Plant Pathology from Cornell University in 1929 ; joined Cornell University as Assistant Professor (1929), became Professor in 1936, Chief of Research in Plant Pathology Division, New York Agricultural Experiment Station, Geneva (1936) ; Guest Professor Horticultural Research Institute, University of Bristol, England (1936-37) ; joined as Chief of Department of Plant Pathology and Botany, Connecticut Agricultural Experiment Station in July 1939, and became Director in 1948, the position which he occupies today.

His research has been basic to the eventual worldwide use of many commercial fungicides such as cuprous oxide, chloronil, nabam, zineb, maneb and karathane.

Elected to the American Academy of Arts and Sciences in 1951 and National Academy of Sciences in 1953. Received Doctor of Science degree (*honoris causa*) from Vermont University in 1958, and from University of Turin in 1964. Author of several research papers and two books—Fungicides and their action and Principles of fungicidal action.

Co-editor with Dr. A. E. Dimond of 'Plant Pathology', an advanced treatise published in 1959-60 ; member of several scientific and honor Societies within the country as well as abroad, including Indian Phytopathological Society since 1948. President, American Phytopathological Society in 1951. He is one of 30 members of National Advisory Commission on Food and Fiber, appointed by President of U. S. A. in 1965.

Teikichi Fukushi

Born on September 5, 1894, at Hakodate City, Hokkaido, Japan ; graduated from the Faculty of Agriculture, Hokkaido University in 1919 ; Professor of Botany and Plant Pathology, Tottori College of Agriculture (1921) ; visited United States of America and the continent (1925-27) ; Assistant Professor of Botany and Plant Pathology, Hokkaido University (1929) ; received Doctorate degree in Agriculture in 1934 ; promoted Professor of Plant Pathology, Hokkaido University (1946) ; retired from Academic service 1958.

Dr. Fukushi is recipient of several honours in recognition of his work. He was awarded the Japan Agriculture Prize for the studies on rice dwarf disease in 1941. On retirement from service in 1958 he was made Professor emeritus. In 1964 he was elected to membership of Japan Academy and was also appointed as Counsellor of Plant Virus Research Institute of Japan.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

J. F. DASTUR

Born on September 10, 1886, at Bhavnagar; M.Sc. Bombay University (1919), D. I. C., London (1920); entered government service in 1910 as First Assistant to the Imperial Mycologist; promoted to Imperial Agricultural Service in 1919, Mycologist to Central Provinces Government from 1923 to 1941, appointed as Imperial Mycologist and Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute., New Delhi, for two years (1945-47).

Studies on the genus *Phytophthora*, cotton wilt, foot rot of pan, ripe rot of bananas, stem breaking of cotton, foot rot and black point of wheat, black thread of *Hevea*, anthracnose and *Namatospora* of cotton and pink disease of citrus are some of his contributions to science.

He is a Fellow of National Institute of Sciences (F. N. I.), and a founder member of Indian Phytopathological Society and First President of I. P. S. Beside departmental leaflets and bulletins he has published about 36 original papers and memoirs in various scientific journals. He has also published four text books.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CONSPECTUS OF OUR KNOWLEDGE IN THE GENERA OF USTILAGINALES*

M. J. THIRUMALACHAR

Hindustan Antibiotics Research Centre, Pimpri, Poona

The Ustilaginales or smut fungi include genera and species parasitising some of the most important food crops of mankind. The early history of our knowledge of smut fungi and the economic importance of successful control of smuts in crop production have been reviewed in several monographs and books. Tillet's studies on the bunt or stinking smut of wheat in 1775, the work of Prévost in 1807 who demonstrated the efficacy of copper compounds in the control of wheat bunt and Jensens' hot water treatment for control of loose smut of wheat are really classical. The enormous loss incited by smut fungi for the bread-basket of mankind stimulated keen interest of mycologists in this group, and various studies on the morphology, physiology, cyto-genetics, taxonomy and control have greatly enriched our knowledge. Among the early pioneers, names of Tulasne brothers, Brefeld and de Bary stand out most prominently. In the last 50 years, the contributions in the study of smuts with reference to different aspects are so many, that it is best to refer to the bibiliography given in the recent book by Fischer and Holton (1957).

I have chosen the topic on the Conspectus of our knowledge in the genera of Ustilaginales as an example to show, how in spite of there being so much of work on this group, there is still greater need for more critical work. In the short period at my disposal I would give briefly a conspectus of the main generic characters as understood at present, also indicating some of the new data, which have been collected in my own studies of the genera of smut fungi in the last several years. In this connection I would like to mention my gratitude to late Dr. B. B. Mundkur, who first initiated me into the studies on smut fungi, my first paper being the new smut genus *Mundkurella*, named in his honour.

The nomenclature of smut fungi starts from December 31, 1801, with Persoon's *Synopsis Methodica Fungorum* as the starting point, for Ustilaginales, together with Uredinales and Gastromycetes. The phylogeny of smuts has been quite controversial, and the present view that they should be near Auriculariaceae and nearer Uredinales is becoming generally accepted. Bessey's terminology of calling the smut spores as teliospores, to indicate their resemblance to rust fungi, is also widely used.

There are usually two well-known families recognized under Ustilaginales, the Ustilaginaceae and Tilletiaceae. These were set up by Tulasne, who first carried out germination studies in 1847. They used

*Presidential Address for 1965 delivered at the meeting of Indian Phytopathological Society held on 2nd January, 1966 at Chandigarh.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the name promycelium for the germ tube, which is continuous, bearing terminal sporidia in case of Tilletiaceae, and septate, with lateral and terminal sporidia in Ustilaginaceae. Fischer and Holton (1957) following the views of Cunningham (1924) have tried to recognise only one family, Ustilaginaceae on account of the fact that some of the smut fungi included in either families show irregularities. Some of the members of the Tilletiaceae develop septate promycelium, and in some of the Ustilaginaceae, the spores germinate directly into infection hyphae. This concept is not correct, since when we take a large number of genera and species whose teliospore germinations have been known, the distinction of the two families is quite apparent. The development of septa in the promycelia of Tilletiaceae is a result of septations to separate the empty portions of the promycelium after the spore contents have migrated upwards. These septa do not differentiate promycelium into cells from which sporidia or infection hyphae are formed. Even so, in many of the Ustilaginaceae, though sporidia may not be formed, the promycelial cells behave as sporidia by developing conjugation tubes. Much of the confusion has arisen since many of the investigators have used different media for spore germination. Under the influence of nutritive conditions, moisture and temperature, the proliferation of the sporidia or promycelial cells frequently occurs. False impressions of a terminal cluster of sporidia in an *Ustilago* are often figured. The basic types of germination, either as in Ustilaginaceae or Tilletiaceae are constant, and some of the abnormalities that may occur should not be overstressed. For instance in *Pericladium grewiae* Pass. the promycelium is septate bearing a single terminal sporidium, while in *P. tiliacearum* Mundk. and Thirum., the septate promycelium develops both terminal and lateral sporidia. This is similar to some of the *Endophyllum* species among rusts, where the number of sporidia is a reflection on the type of aberrant nuclear cycle. The basic type of germination in *Pericladium* however is as in Ustilaginaceae. The distinction of families Ustilaginaceae and Tilletiaceae, first recognised by Tulasne in 1847, is well founded.

While the main families are recognized on the basis of spore germination, the genera are based on the characters of the sori and spore morphology. It is true that spore germination for a large number of species has not been determined, but this should not prevent us from grouping the characters where they are known. The spores may be single, united in pairs or spore balls, or multicellular structures. Taking the Ustilaginaceae first, the distinguishing characters of the genera are given below. The synonyms are not discussed here.

(1) *Ustilago* (Pers.) Roussel in *Flore du Calvados* p. 47, 1806.

Sori in various parts of the host, such as leaves, stems and fruits, black and dusty at maturity. The sorus is covered by the host tissue. The spores germinate in various ways, but in main, conforming to the type in Ustilaginaceae.

(2) *Pericladium* Passerini emend Mundkur in *Mycologia* **36**, 291, 1944.

The genus is characterised by the formation of gall-like sori, in which there are locules. The wall of the sori is composed entirely of host tissue, and the agglutinated dark spore mass is present inside.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The spores are unicellular and germinate as in *Ustilago*. In *Pericladium grewiae*, the spores germinate by a septate promycelium and a single terminal sporidium while in *P. tiliacearum* there are both terminal and lateral sporidia (Thirumalachar, 1950). The sporidia have been grown in artificial culture, and resemble other smuts in general. Ciferri (1953) who examined some herbarium material of this genus stated that *Pericladium* is a member of the Fungi Imperfecti, which is incorrect. The genus *Xylosorium* described by Zundel (1939) was shown by Mundkur to be synonymous with *Pericladium*.

(3) *Melanopsichium*. Beck in *Ann. K. K. Natur. Hofmus. Wien.* **9**, 122, 1894.

This genus is characterised by the formation of chambered sori which are formed by the development of lysigenous cavities within the host tissue. The hyphae bordering the sorus secrete a gelationus matrix, in which the spores are embedded, and often extruded out as spore tendrils. The presence of chambered sori and spores embedded in gelatinous matrix are characteristic. The spores germinate as in Ustilaginaceae.

(4) *Liroa* Ciferri in *Nuovo Gior. n. Bot. Ital. N. S.* **40**, 26, 1933.

The genus was first described by Ciferri by transferring *Ustilago emodensis* under the new genus. The distinguishing characters as given by Ciferri were not convincing. He had included *Ustilago nepalensis* also under that genus. Mundkur and Thirumalachar (1946) who restudied the genus showed that the differentiation of a large sterile tissue and an apical lunate bed of fertile sorus opening out by pores at apex is the most important character. The sorus is traversed by strands of host and mycelial tissues in the form of trabiculae. The spore germinations are as in *Ustilago*. Ling (1949) commented that this type of structure seen on the shoots, is not seen when fruits are infected. Evidently he did not examine well-preserved material, since on the fruits also, the type of sori formed is the same as on the shoots. Many of the workers on smuts who have not examined good collections of *Liroa emodensis* prefer to use the name *Ustilago emodensis*, which is not correct. The genus is well differentiated from others by the morphology of the sorus.

(5) *Sphacelotheca* de Bary in *Verg. Morph. Biol. Pilze.* p. 187, 1884.

This genus described by de Bary is differentiated from *Ustilago* by the possession of an outer false membrane covering the sorus, and a central columella of host tissue. The false membrane is composed of fungus cells which are transformed spores. In many cases the false membrane is evanescent, and the smut is mistaken for a species of *Ustilago*. Examination of a young sorus provides the necessary diagnostic characters.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(6) *Franzpetrakia* Thirumalachar & Pavgi in *Sydowia*, Festschrift für Franz Petrak, Beiheft, **1**, 1-3, 1957.

Sori covered with host membrane with central columella of host tissue. Sterile filamentous hyphae spirally coiled and functioning as elaters attached to the columella and free at the other end, spores exposed on the brush-like mass of elaters at maturity. Spore germination as in Ustilaginaceae.

(7) *Cintractia* Cornu in *Ann. Sci. Nat. Bot. Ser. VI.* **15**, 279, 1883.

The genus is based on the type *C. axicola* (Berk.) Cornu which usually attacks the peduncles and pedicels of various *Fimbristylis* species in the world. Thirumalachar (1950) has given a detailed account of the sorus morphology of *Cintractia* and its relation to *Farysia* Racib. The sorus is formed centripetally round a columella, with bands of fertile and sterile hyphal strands alternating and producing centrifugally spores which are surrounded by the false membrane and the host layers. The spores of *C. axicola* have never been germinated before, and Thirumalachar (unpublished) showed that it is 2 to 3-septate, bearing lateral and terminal sporidia. There has been considerable confusion in identification of *Cintractia*. Species in ovaries of cyperaceous hosts, with agglutinated spore mass and false membrane, which are true *Sphacelotheca*, are misdetermined as *Cintractia*. Some of the species have been described on leaves. These and many others described as *Cintractia* from North America have to be re-examined. In this connection the genus *Planetella* of Savile (Canad. Jour. Bot. **29**, 326-27, 1951), which is just based on the character of the thickening of the spore along the equatorial region, has to be considered. Partial thickening of spore wall is well-known as in loose smut of wheat, and occurrence of hyaline polar caps is known in several others like *Cintractia scleriae-elatae*. Thirum., *C. montagnei*. Magnus, etc. The latter species has the same spore structure, with thickening of spore wall in the centre. This cannot be given generic status. *Planetella* lacks the sterile bands of hyphae of *Cintractia*, and has to be considered as synonym of *Sphacelotheca* (Thirumalachar, unpublished). The genus *Anthracoidea* Bref. has been revived, since it produces a two-celled promycelium with sporidia, and the germination in *C. axicola* the type of the genus was unknown. Now *C. axicola* is shown to produce a 3-4 celled promycelium with sporidia. A separate genus for accommodating species with 2-celled promycelium appears to be unwarranted.

(8) *Farysia* Raciborski in *Bull. Acad. Sci. de Crac. CI. Math. Nat.* 1909, 354, 1909.

The sorus is covered by an outer false membrane, and opening out by numerous brush-like elater-like hyphae, with dusty spore mass. Thirumalachar (1952) has shown that *Farysia* structurally is identical with *Cintractia*, but the plane of development instead of being around a columella, is vertical, at the apex of the thalamus. The sterile strands of hyphae traversing through the spore mass in *Cintractia*, appear like brush in *Farysia*.

(9) *Cintractiella* Boedijn in *Bull. Jard. Bot. Buitenzorg Ser. III.* **14**, 368, 1937.

This remarkable smut, *C. lamii* Boedijn, incites development of small adventitious shoots on the leaves of *Hypolytrum*, with an elongated

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sorus surrounded by a false membrane. The chief character of the genus is the presence of a germ pore. Examination of some of the material kindly obtained from Dr. Boedijn showed that the spores with germ pore had no spore contents, and it looked like spores where the promycelium had developed and collapsed, leaving a wide distended pore. This point needs reconfirmation.

(10) *Schizonella* Schroeter in *Cohn. Beitr. Biol. Pflanz.* **2**, 362, 1877.

The sori are in leaves, the spores are two-celled by the internal septation of the spore mother cell. Germination as in Ustilaginaceae.

(11) *Schroeteria* Winter in *Rabeh. Krypt. Flora* **1**, 117, 1881.

Sori in seeds, dark and powdery at maturity, spores in pairs, yellowish-brown, germinating at maturity by septate promycelium bearing lateral and terminal sporidia.

The type of the genus, *Schroeteria delastrina* (Tulasne) Winter, parasitises the ovaries of *Veronica praecox* (Scrophulariaceae). The spore germination was given by Brefeld, which showed hyphae emerging out, and developing chains of spores at the tip. There was considerable difference of opinion about the position of the genus in the Ustilaginaceae or Tilletiaceae. At persent, the genus is placed under Tilletiaceae. Material from Rumania became available through the kindness of late Dr. T. Savulescu. The spores germinated by stout promycelium, septate, bearing lateral and terminal sporidia. The spore wall was widely ruptured. All indications show that Brefeld's material, which had formed chains of conidia had an endophytic Hyphomycete. The genus *Schroeteria* is a member of the Ustilaginaceae, nearer to *Schizonella*. It is difficult to separate the two on the basis of slight differences in spore shape, and the genus has been merged with the earlier described *Schizonella* Schroeter by Thirumalachar (in press).

(12) *Mykosyrinx* Beck in *Ann. K. K. Natur Hofmus. Wien*, **9**, 123, 1894.

Sori within peduncles and pedicels, with double-layered peridium; spores developed in pairs at the ends of sporogenous cells. Germination as in Ustilaginaceae.

(13) *Mundkurella* Thirumalachar in *Mycologia* **36**, 594, 1944.

Sori in stems, petioles and fruits and spores are heterosporous: (1) one-celled, some chocolate-brown and others pale-yellow and hyaline, and (2) two celled spores, chocolate-brown and with rich cell contents. Germination by septate promycelium, with constrictor cells in between.

The genus is monotypic, parasitising *Heptaplerum venulosum*, a member of the Araliaceae. Dr. Moss. and Dr. Diehl have an unpublished species of the same genus on *Aralia* species in Canada. It is hoped that this smut would be described at an early date.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(14) *Sorosporium* Rudolphi in *Linnaea* **4**, 116, 1829.

Sori in various parts of the host, chiefly characterised by the possession of spore balls which are not united to each other by any projections of the outer wall. Spores germinating by septate promycelium bearing lateral and terminal sporidia.

The details of the characteristics of this genus are being published by Thirumalachar and Pavgi (in press). The sori may be covered with false membrane and possessing a columella as in *Sphacelotheca*, or sori as in *Ustilago*. The spore balls may have all spores of one type, or outer spores coloured and the inner ones hyaline, and thus heterosporous. Some of the species are very specific and attack only the anthers producing spore balls.

(15) *Tolyposporium* Woronin in *Abh. Senck. Nat. Ges.* **12**, 577, 1882, emend Clinton, Thirumalachar and Pavgi (in press).

The sori are as in *Sorosporium*. The spore balls may be composed of same type of spores or the outer layer has coloured and the inner layer hyaline spores. The main differentiating character is the development of outgrowths from the outer wall of the spore, which intercalate and articulate with those of the neighbouring spore and thus forming the spore ball. The spore germination is as in Ustilaginaceae.

The type of the genus proposed by Woronin is *Sorosporium junci* (Schoret) Woronin on *Juncus bufonius*. An examination of most of the collections by Thirumalachar and Pavgi (in press) has shown that it is a *Sorosporium* species. The permanency of the spore ball has no meaning since it is determined by the age of the material and the pressure employed to break the spore ball. The characters given by Clinton (1906) in North American Flora for the genus are more accurate. The articulation of the projections of the exospore was pointed out by Clinton. Since the type of *Tolyposporium* is a *Sorosporium*, the usual procedure is to reject the genus and propose a new name for those having characters as described above. Since the name is well established, Thirumalachar and Pavgi proposed to retain the name *Tolyposporium* and designated another type.

(16) *Ustacystis* Zundel, *Mycologia* **37**, 796, 1945.

The genus was proposed by Zundel to accommodate a smut on *Waldsteinia fragroides* with spore characters as in *Urocystis*, but germinating by septate promycelium and lateral sporidia. It is therefore, a member of the Ustilaginaceae.

(17) *Thecaphora* Fingerhuth *Linnaea* **10**, 230, 1836

The sori are formed in various parts of the plant, developing spore balls. The spore balls are actually muticellular spores, and a comparative morphology of the spore development in *Sorosporium*, *Tolyposporium* and *Thecaphora* has confirmed this feature (Thirumalachar, in press). Fischer and Holton state that separation of these genera is a difficult matter. It does not appear so, since in *Sorosporium* and *Tolyposporium*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the spore ball initial is composed of hyphae, balling up, and the individual hyphal cells rounding off after gelatinisation. In *Thecaphora* on the other hand, the spore mother cell undergoes septation in different planes and forms the muticellular spore. The inner wall for two adjacent spores is common, thus confirming the multicellular condition brought by septation. The germination in *Thecaphora* is stated to be by the formation of a single terminal sporidium. However in some of the species like *T. aterrima* var. *fimbristylidis*, Thirumalachar noticed the development of lateral sporidia also. Since the genus is well characterised by the development of multicellular spores, the germination type based on the number of sporidia need not be a diagnostic character.

(18) *Tolyposporella* Atkinson in *Bull. Cornell Univ.* 3 (1) 16, 1897.

The genus as described by Atkinson is vague, and several of the other species included therein by later workers do not belong to that genus (Thirumalachar and Whitehead, 1966). The main character in *T. chrysopogonis* Atkinson, the type of the genus, is the formation of spore balls, ephemeral in nature, formed by the gelatinisation and coalescence of the outer walls of the spore. The occurrence of a single lateral sporidium on a septate branched promycelium is not a constant character. The other species included are not *Tolyposporella* species, and one of them *T. obesa* has been shown to belong to a genus of the Tilletiaceae, *Jamesdicksonia* Thirumalachar *et al.*

(19) *Dermatosorus* Sawada *apud* Ling in *Mycologia* **41**, 267, 1949.

The sori are formed in ovaries, consisting of spore balls which are covered by a thick dark pseudomembrane. The spore balls have outer fertile spores and inner core of delicate pseudoparenchymatous cells. Germination is unknown and hence its position under Ustilaginaceae is tentative.

(20) *Testicularia* Klotz. in *Linnaea* 7, 202, 1832.

The genus is characterised by the development of large spore balls covered by a thick peridium-like pseudomembrane. The spore balls have a single outer layer of fertile spores and a central mass of sterile parenchymatous cells ; germination unknown. Though externally the sori appear different, in general structural characters it closely resembles *Dermatosorus* Sawada, which is a later described genus. The species in both of the genera parasitise members of Cyperaceae, developing spore balls. It seems advisible to keep them separate until spore germinations are known.

(21) *Zundelula* Thirumalachar & Narasimhan in *Sydowia* **6**, 409, 1952.

Sori in ovaries, enveloped by thick pseuodoperidium of interwoven hyphal strands enclosing large spore balls which are permanent and black. Outer two or three layers of dark coloured cells are sterile and fragile, and inner core of fertile spores which are yellowish-brown, interconnected by webbing of yellow pseudoparenchymata. Germination by septate promycelium bearing lateral and terminal sporidia.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(22) *Kweilingia* Teng in *Sinensia* **11**, 105-130, 1940.

The sori are formed on the leaves as in *Jamesdicksonia*, but the spores germinate by 3-septate promycelium bearing lateral and terminal sporidia. *Kweilingia bambusae* was first described as a rust, under the name *Chrysomyxa bambusae*, and later transferred under a new genus. After the detailed work on *Jamesdicksonia*, Thirumalachar *et al.* (1960) concluded *Kweilingia* also has the same sorus characters as *Jamesdicksonia* and only differs in having spore germination as in Ustilaginaceae.

TILLETIACEAE

(1) *Tilletia* Tulasne in *Ann. Sci. Nat. Bot. Ser.* 7, 112-113, 1847.

Sori are mostly ovaricolous and occasionally in other plant parts also. Spore mass associated with sterile cells, and spores more than 15μ in diameter, germinating by septate or unseptate promycelium bearing a terminal whorl of sporidia. Ciferri tried to restrict the genus to ovaricolous smuts, but several of them like *Tilletia tumefaciens* Syd. on *Panicum antidotale* occur on culms. The number of sporidia produced was made the basis for separation of the genus at one time, those developing large number of sporidia being placed under *Neovossia* Koern. Fischer and Holton attempted to restrict *Neovossia* to species possessing a small hyphoid appendage or apiculus, which is a remnant of mycelium left behind after the gelatinisation of the sporiferous hyphae. If immature sori of *Tilletia* are teased out, this apiculus is often seen though not present in old sori. *Tilletia sphagni* Nawaschin, was described in the capsules of *Sphagnum*. This was worked out in detail by Bauch, who showed that it is the conidial form of *Helotium* species, one of the Discomycetes. Ciferri however, created a new genus for it, *Bryophytomyces*, which does not appear to be a valid one.

(2) *Neovossia* Koern. in *Oesterr. Bot. Zeit.* **29**, 217, 1879.

This genus is restricted to forms of *Tilletia*, showing the distinct apiculus and developing numerous sporidia, which usually do not conjugate *in situ*. Only three species are recognized by Fischer and Holton, *N. iowensis* in U.S., and *N. danubialis* and *N. moliniae* from Europe.

(3) *Entyloma* de Bary in *Bot. Zeit.* **33**, 101, 1874.

Sori formed in various plant parts, mostly in leaves, the spores are intercellular, hyaline or pale-yellow, germinating as in Tilletiaceae. Some of the species develop secondary conidial stages which resemble the Fungi Imperfecti, *Cylindrosporium*. The conidia are extruded out of the stomata. Several of the species like *E. oryzae* Syd. or *E. lineatum* (Cke) Davis and others possess dark coloured spores, and if this is the real basis for differentiating *Melanotaenium* from *Entyloma*, several of these species should properly be transferred under *Melanotaenium*.

(4) *Melanotaenium* de Bary in *Bot. Zeit.* **32**, 105, 1874.

Sori as in *Entyloma*, some of them forming galls or dark patches; spore mass agglutinated, indehiscent, spores germinating as in Tilletiaceae. There are large number of species developing dark sori on stems, and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

some like *M. euphorbiae* (Lenz) Thirumalachar and Whitehead form conspicuous galls.

(5) *Entorrhiza* Weber in *Bot. Zeit.* **42**, 378, 1884.

This genus was founded to describe smuts developing galls on roots, producing digitate swellings beneath the soil. The spore mass is compacted together, indehiscent, germinating as in Tilletiaceae. Large number of collections of this smut genus examined by Thirumalachar (in press) indicated that this genus is synonymous with *Melanotaenium*. from which it is at present separated by the position of the sori on roots. This cannot be a generic character, since smuts like *Urocystis coralloides*. Rostr. attack roots of *Brassica* species and a separate genus is not desirable for its accommodation. Thirumalachar therefore proposed merging of *Entorrhiza* with *Malanotaenium* which is also known to produce sori on stems, often inciting swellings.

(6) *Glomosporium* Kochman in *Acta Soc. Bot. Polon.* **16**, 58, 1939.

Sori as in *Thecaphora*, composed of multicellular spores, which germinate as in Tilletiaceae with a cluster of sporidia, which show spicules as appendages. Only Kochman has described the germination in *G. leptideum* (Syd) Kochman, and the appendages in a structure like sporidium are difficult to imagine. These protrusions may be of the nature of conjugation branches. The genus is however characterised by multicellular spore heads of the type of *Thecaphora* and germination as in Tilletiaceae.

(7) *Tuburcinia* Fries in *Syst. Mycol.* 3, 439, 1829, emend Woronin, *Abh. Senck. Nat. Ges.* **12**, 561, 1882.

Sori in various parts of the host, developing permanent black spore balls. All the spores are dark and fertile and germinate as in Tilletiaeae The development of secondary conidia through the stoma is also known, in some species. Liro (1922) who examined the type of *Tuburcinia* found that it was a species of *Urocystis* and therefore transferred all *Urocystis* species under *Tuburcinia*. The International Botanical Nomenclatural group have conserved the name *Urocystis* for those having spore balls with sterile cells. A similar situation has arisen in *Tolyposporium*, the type of which, *T. junci*, is a *Sorosporium*, and Thirumalachar and Pavgi have recommended conservation of the name *Tolyposporium*. Ciferri proposed the name *Ginaniella* for those true *Tuburcinia* species which had no sterile cells. This is unnecessary since the name *Tuburcinia* has been retained for those species with spore balls with all fertile spores.

(8) *Urocystis* Rabenhorst in *Klotzsch Herb. Viv. Myc. LL.* No. 393, 1856.

Sori in various parts of the host including roots, developing spore balls. Fertile spores dark coloured and associated with sterile cells. Germination as in Tilletiaceae.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(9) *Doassansia* Cornu in *Ann. Sci. Nat. Bot. Ser. VI*, **15**, 285, 1883.

Sori are developed in leaves and other parts of the host, pale-yellow, immersed in host tissue. The spore balls are hyaline to pale yellowish-brown and composed of an outer sterile cortex and inner core of fertile spores which germinate readily as in Tilletiaceae.

(10) *Doassansiopsis* (Setchell) Diet. in *Nat. Pflanzenf.* **1**, 21, 1897.

The genus is differentiated from *Doassansia* by the development of a single outer layer of fertile spores, surrounded by sterile hyphal cells or hyphal sheath, with an inner core of sterile parenchymatous cells. Germination as in Tilletiaceae. Fischer and Holton, Zundel and several others failed to appreciate and recognise the basic distinguishing characters of *Doassansiopsis*, and treat it as a synonym of *Doassansia*. Fischer and Holton treat it as synonym of *Burrillia*. If one considers the situation in *Testicularia* where the single outer layer of fertile spores is the chief differentiating character and *Tracya*, where the lack of outer sterile layer and the presence of a single fertile spore layer are the chief characters, one cannot overlook the important sorus structure of *Doassansiopsis*, which is a valid genus and different from *Doassansia* or *Burrillia*.

(11) *Burrillia* Setchell in *Proc. Amer. Acad. Arts Sci.* **26**, 18-19, 1891.

The spore balls are as in *Doassansia*, but lacking sterile outer layer, being composed of either all fertile spores or intermixed with sterile cells. Germination is as in Tilletiaceae.

(12) *Tracya* Syd. in *Hedw. Beibl.* **40**, 3, 1901.

Spore balls as in *Burrillia* without any sterile cortex. Fertile spores restricted to a single outer layer, with an inner sterile core of filamentous cells. Germination as in Tilletiaceae.

(13) *Narasimhania* Thirumalachar & Pavgi in *Sydowia* **6**, 390-391, 1952.

Sori as in *Burrillia*, but the spore ball construction being clathroid, with fertile spores interconnected and traversed by yellowish-brown sterile parenchymata. Teliospore germination as in Tilletiaceae.

(14) *Polysaccopsis* Henn. in *Hedw.* **37**, 206, 1898.

This monotypic genus on *Solanum* species in South America incites formation of galls in which there are large number of cavities containing spore balls similar to those in *Urocystis*. No spore germination has been observed

(15) *Jamesdicksonia* Thirumalachar, Pavgi & Payak in *Mycologia* **52**, 478, 1960.

Sori in the leaves, pustulate and erumpent, developing flabelliform crusts with spores arranged in chains. Mature spores germinating by a promycelium bearing a cluster of sporidia as in *Entyloma*.

The type of the genus *J. obesa* was first identified by Sydow and Butler in India as *Entyloma obesa* parasitising the leaves of *Dichanthium annulatun*. This was later transferred under *Tolyposporella* by Clinton and Zundel. The structure of the sorus as flabelliform

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

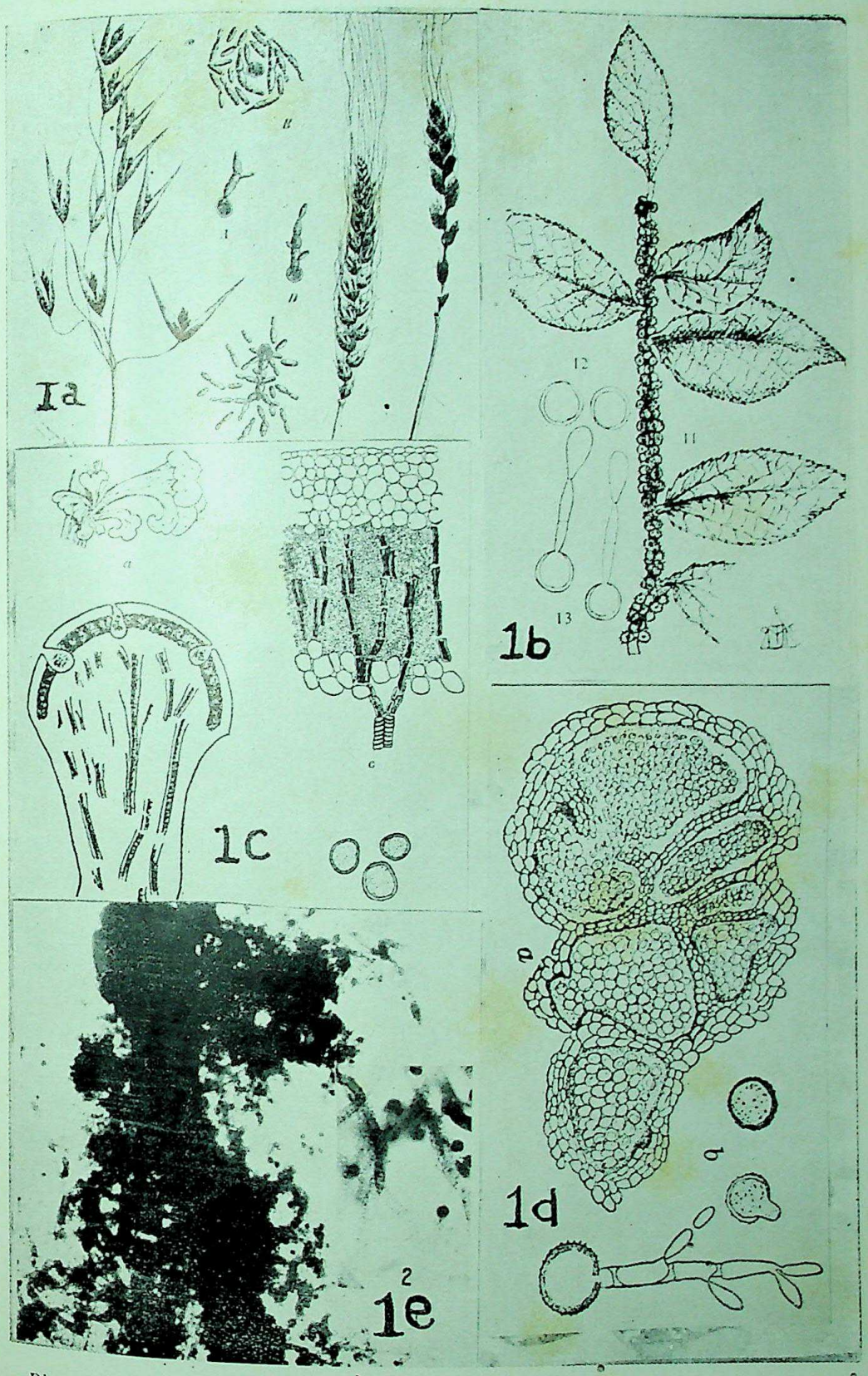


Plate 1. 1a. *Ustilago*, 1b. *Pericladium grewiae*, 1c. *Eiroa emodensis*, 1d. *Melanopsichium austro-americanum*, 1e. *Franzpetrakia microstegiae*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

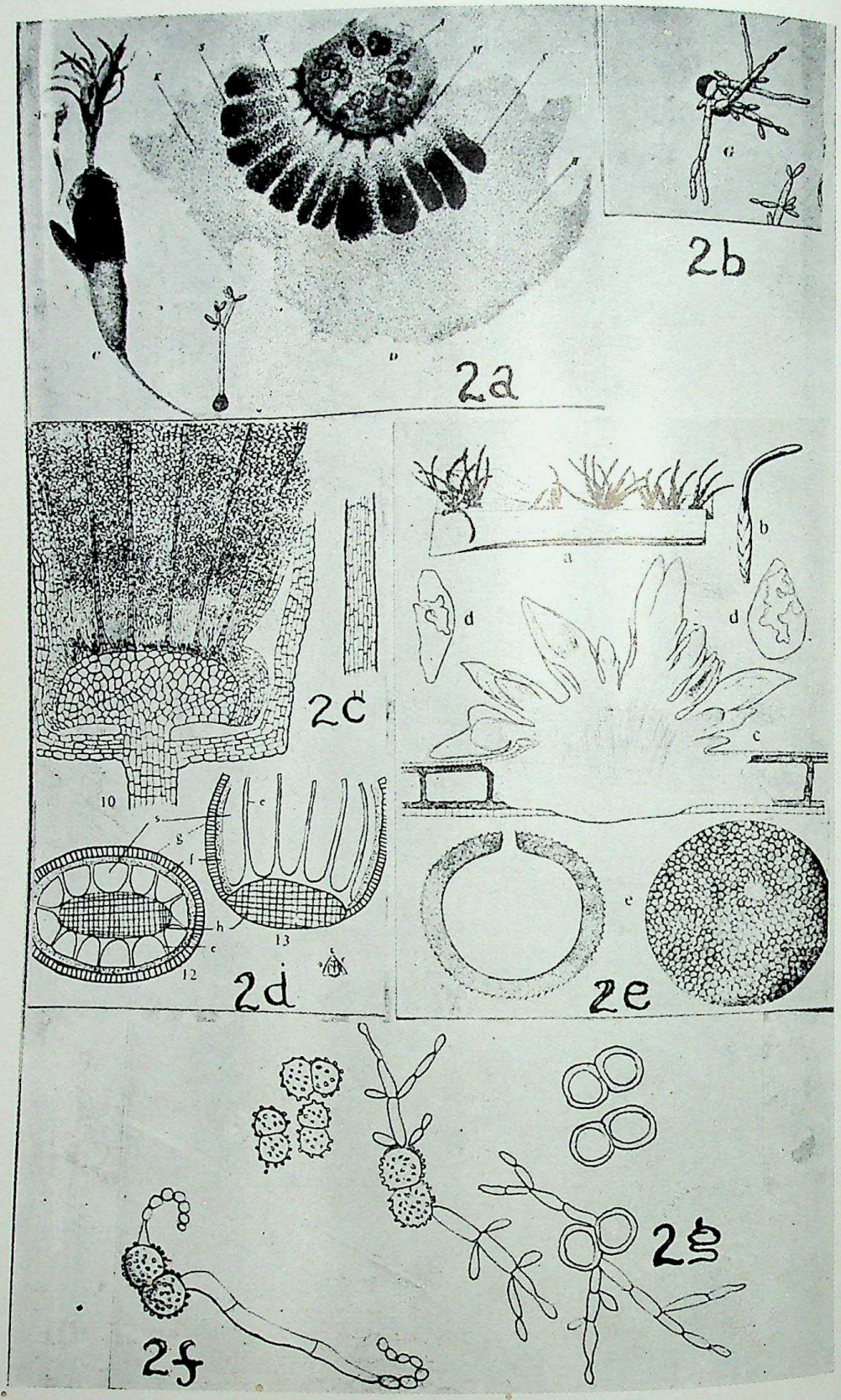


Plate 2. 2a. *Cintractia caricis*, 2b. *Schizonella melanogramma*, 2c. Sorus structure of *Farysia*, 2d. Sorus structure of *Cintractia* 2e. *Cintractiella lamii*, 2f. *Schroeteria delastrina* (germination of spores figured by Brefeld), 2g. *Schroeteria delastrina* (germination in present studies)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

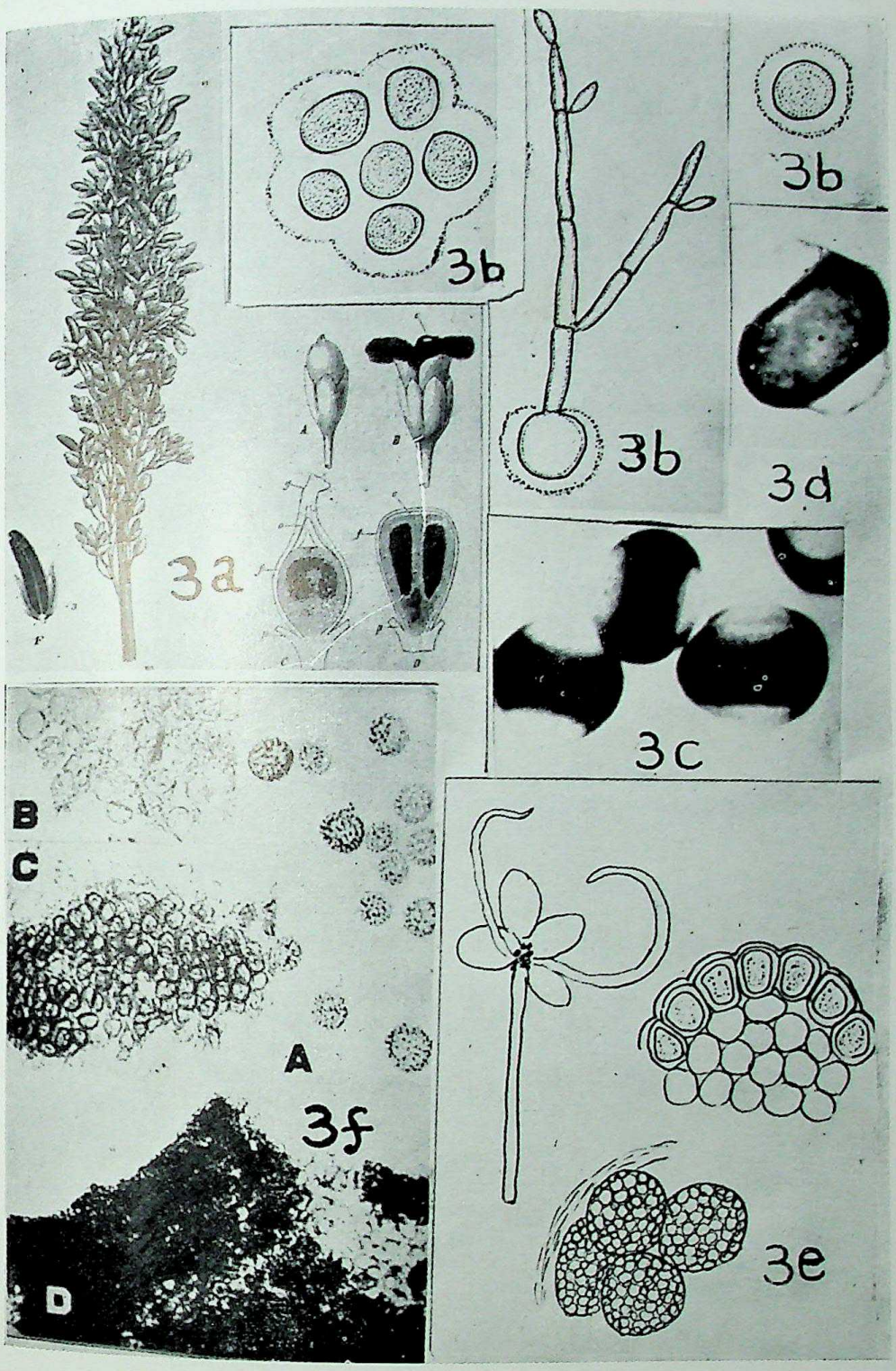


Plate 3. 3a. *Sphacelotheca*, 3b. *Tolyposporella chrysopogonis*, 3c. *Planetella lironis*, 3d. *Cintractia montagnei*, 3e. *Testicularia cyperi*, 3f. *Dermatosorus eleocharidis*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

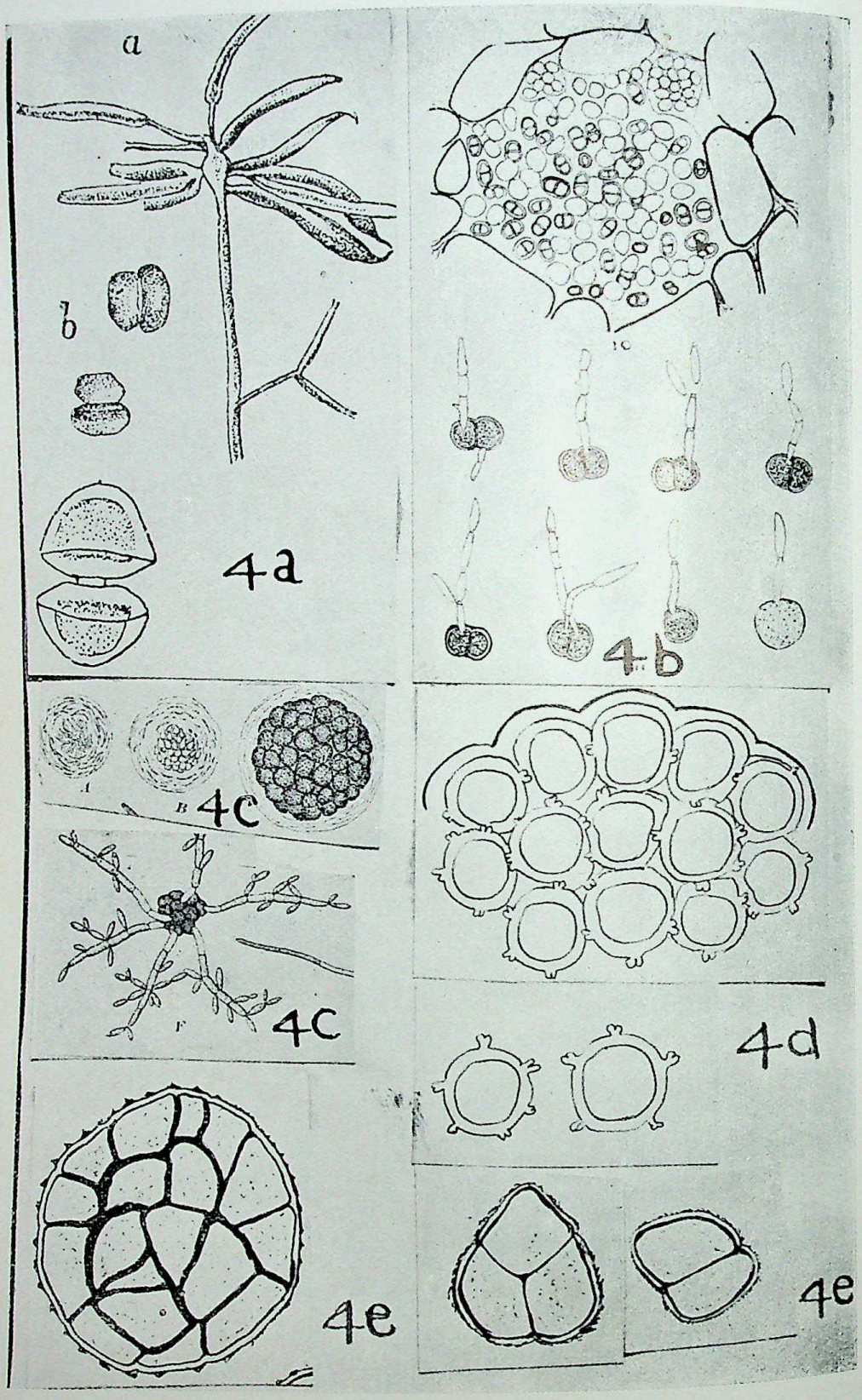


Plate 4. 4a. *Mykosyrinx cissi*, 4b. *Mundkurella heptapleuri*, 4c. *Sorosporium* 4d. *Folyposporium*, 4e. *Thecaphora*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

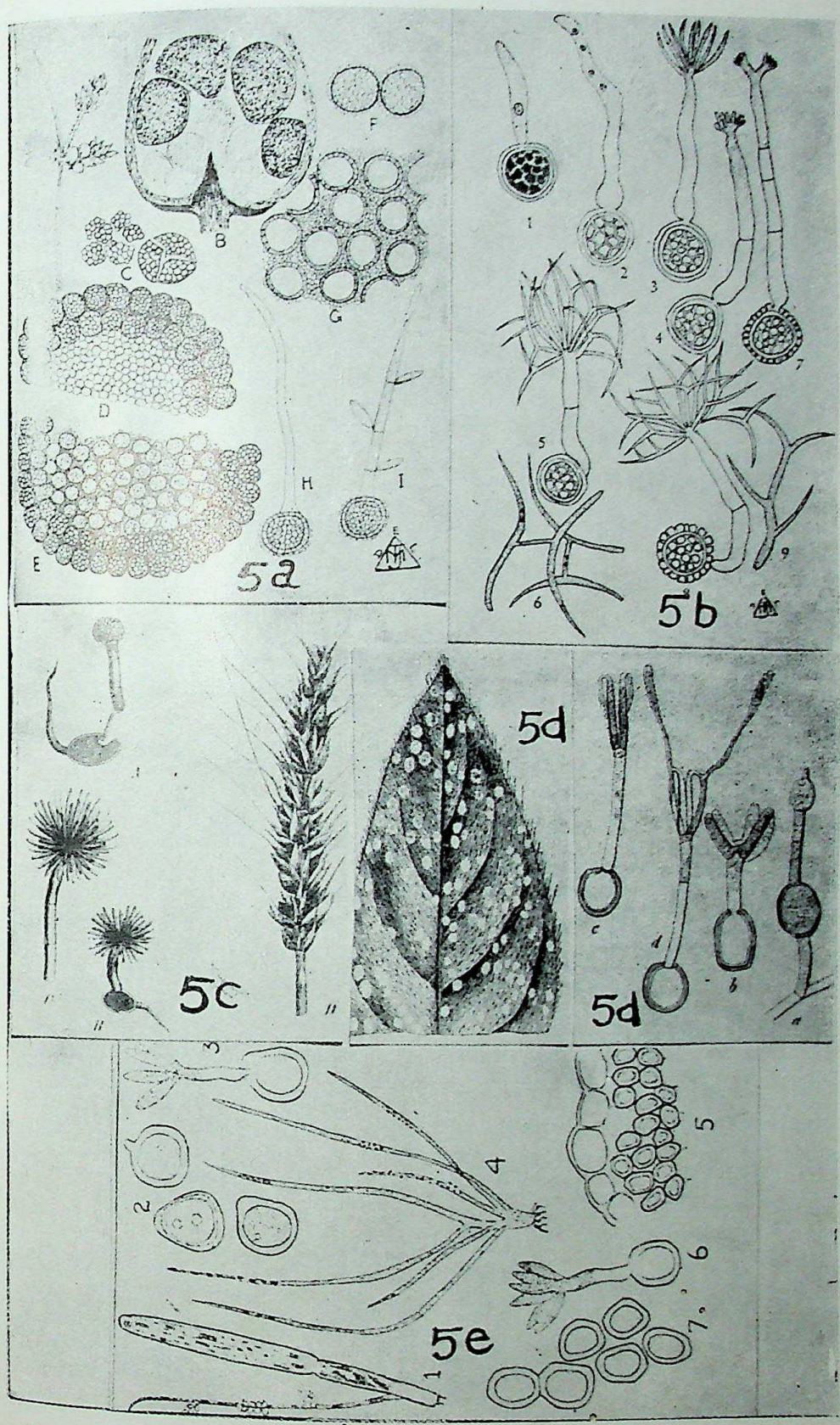


Plate 5. 5a. *Zundelula fimbristylidis*, 5b. *Tilletia*, 5c. *Neovossia*, 5d. *Entyloma* 5e. *Melanotaenium*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

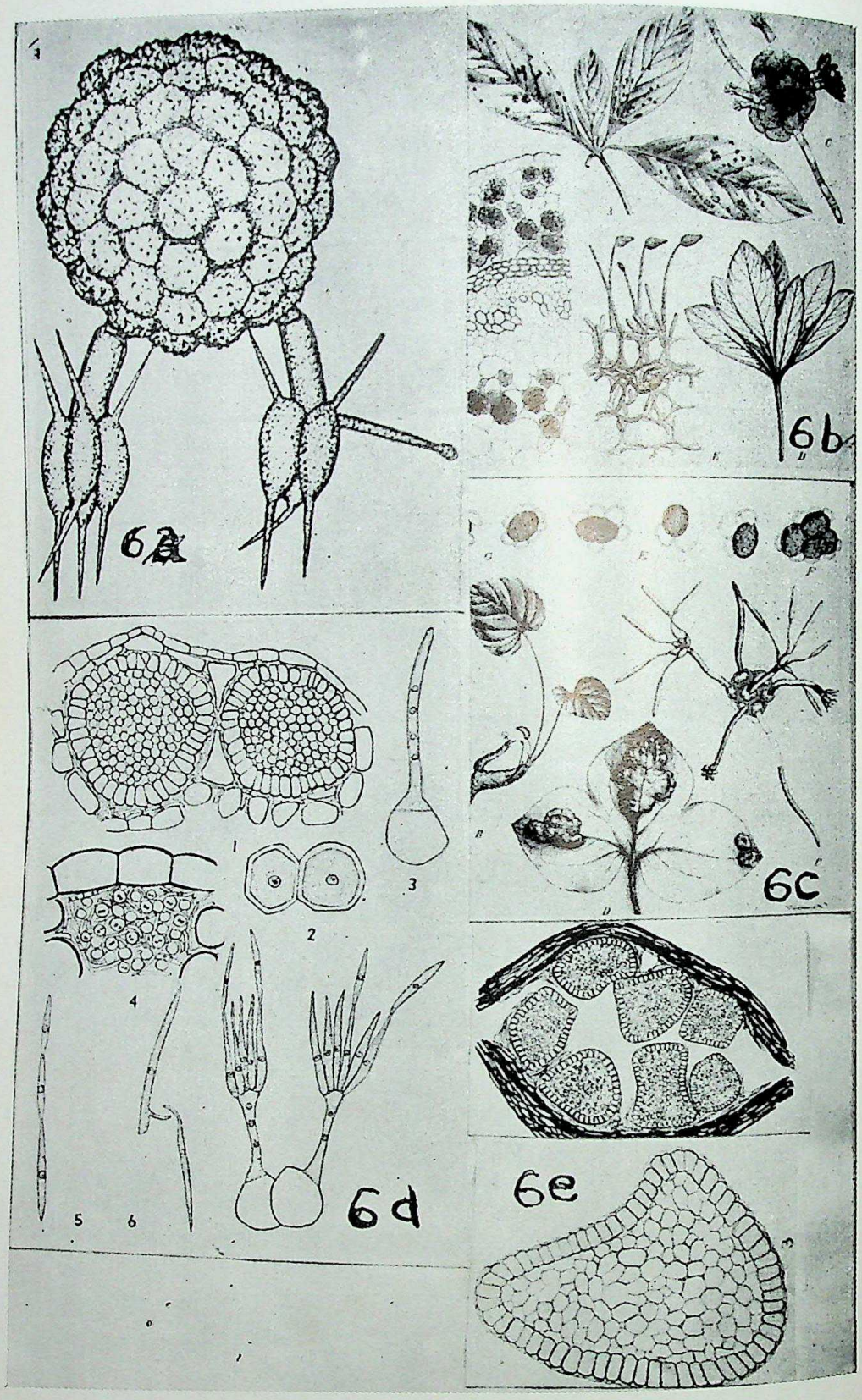


Plate 6. 6a. *Glomosporium leptideum*, 6b. *Tuburcinia trientalis*, 6c. *Urocystis violae* 6d. *Doassansia hygrophilae*, 6e. *Doassansiopsis nympheae*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

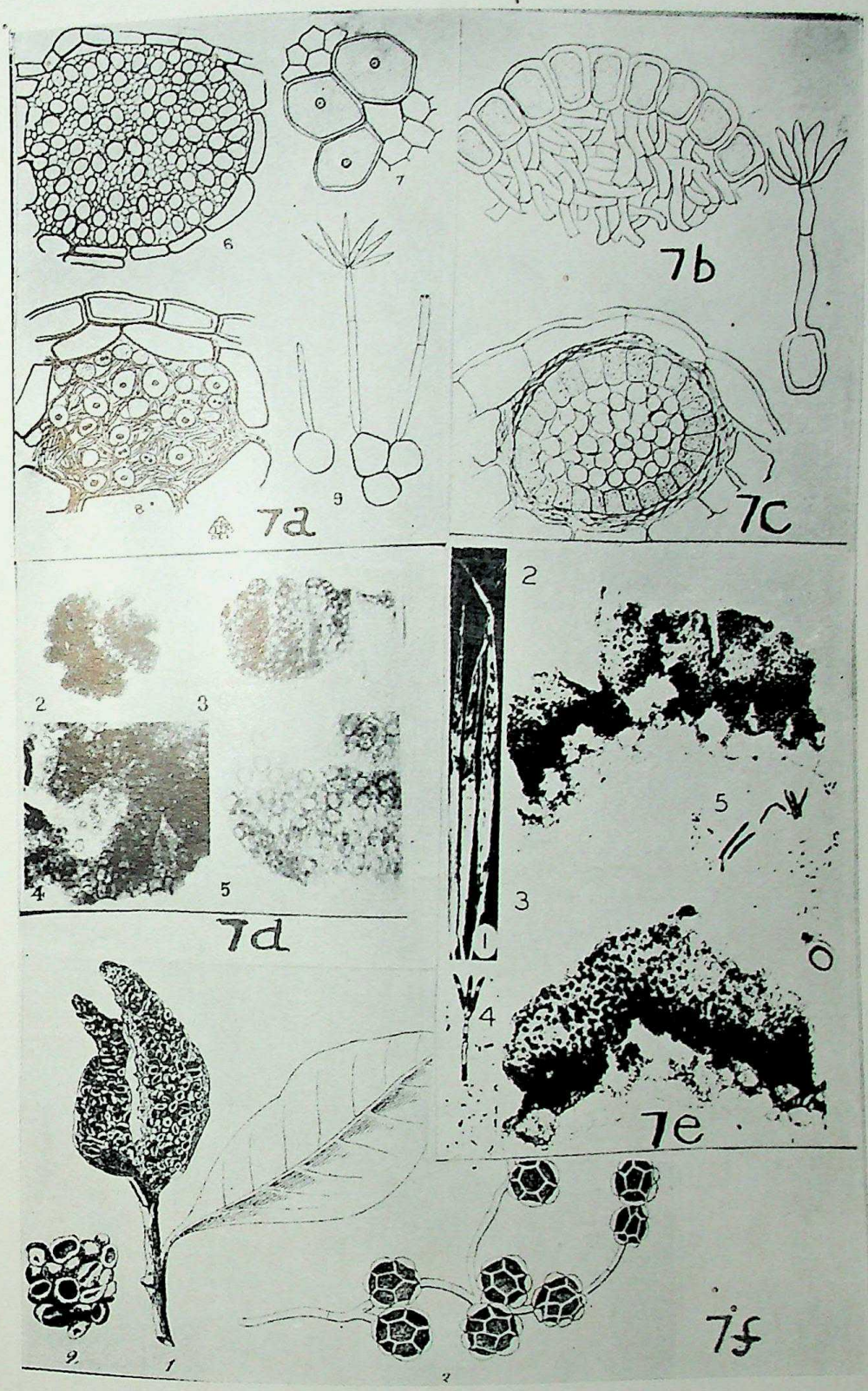


Plate 7. 7a. *Burrillia ajrekari*, 7b. *Tracya lemnae*, 7c. *Tracya hydrocharidis* (=*Doassansiopsis hydrocharidis* (Lagerh) Thirum). 7d. *Narasimhania alismatis*, 7e. *Jamesdicksonia obesa*, 7f. *Polysaccopsis hieronymi*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

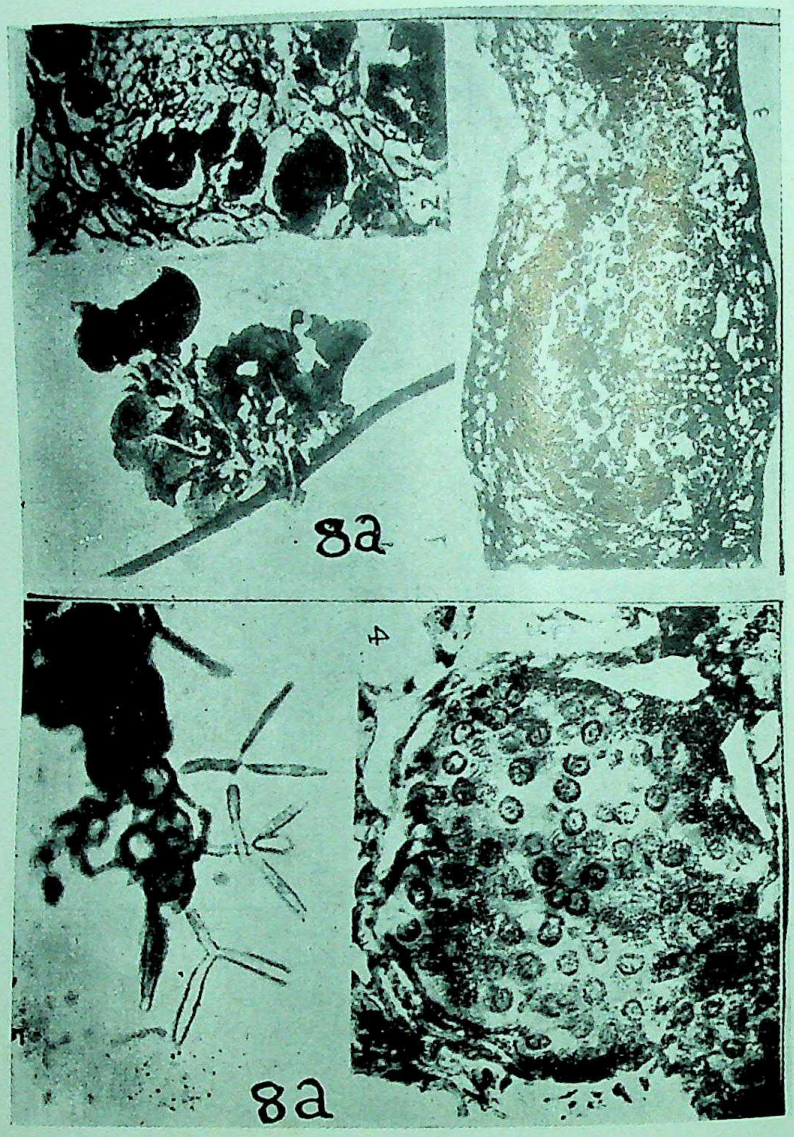


Plate 8. *Georgefischeria riveae.*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

crust is similar in shape to that of *Dasturella*, a genus of rust. These characters together with the type of spore germination as in Tilletiaceae indicated that it represented an undescribed genus.

(16) *Georgefischeria* Thirumalachar & Narasimhan in *Mycologia* 55, 30-34, 1963.

Sori are formed in various parts of the host, usually systemic, developing lysigenous cavities in which spores are embedded in a gelatinous matrix. The mature spores are subhyaline and germinate as in Tilletiaceae. This unique systemic smut *G. riviae* does not show any external infection symptoms, except for smaller leaves and shorter internodes presenting the appearance of a witches' broom. When the leaves are cut and examined, the entire space within the leaf tissue is occupied by the smut sorus. In the organisation of the sorus it resembles *Melanopsichium* and in the spore germination type it resembles *Entyloma*.

Among the excluded genera mention may be made of *Uleiella* Diet, which was considered to be a rust and later a smut. Studies by Thirumalachar (1949) showed that it is neither a rust nor a smut.

References

CIFERRI, R. (1953) Observations on some doubtful genera attributed to smut fungi. *Atti dell Istituto Botanico e Laboratorio crittagamico di Pavia, ser.* 5. **10** : 217-224.

CLINTON, G. P. (1906) "*Ustilaginales*". North American Flora **7** : 1-82.

CUNNINGHAM, G. H. (1924). The Ustilagineae or smuts of New Zealand. *Trans.N. Zeal. Inst.* **55** ; 397-433.

FISCHER, G. W. AND C. S. HOLTON (1957) *Biology and control of the smut fungi* 622 pp. The Ronald Press Co. N. Y.

LING, LEE (1949) A second contribution to the knowledge of the Ustilaginales of China. *Mycologia*, **41** : 252-269.

SAVILE, D. B. O. (1951) Two new smuts on *Carex* in Canada. *Canad. J. Bot.* **29** : 324-328.

LIRO, IVAR (1922) Uber die Gattung *Tuburcinia* Fries. *Ann. Univ. Fenn. Aboensis L. Turku.*

MUNDKUR, B. B. AND M. J. THIRUMALACHAR (1952) *Ustilaginales of India* 84 pp. C. M. I., Kew.

THIRUMALACHAR, M. J. (1950) Notes on some Indian Ustilagineae-I. *Lloydia* **13** : 165-172.

——— ——— (1950) Notes on some Indian Ustilagineae II. *Lloydia* **13** : 173-178.

———, (1949) Critical notes on some plant rusts I. *Bull. Torrey bot. Cl.* **76** : 339-342.

TULASNE, L. R. AND C. TULASNE (1847) Memoire sur les Ustilaginees comparees aux Uredinees. *Ann. Sci. Nat. Bot. Ser. 3*, **7** : 12-127.

ZUNDEL, G. I. (1945) A change in generic name. *Mycologia* **37** : 795-796.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE VIABILITY OF SCLEROTIA OF SCLEROTIUM ORYZAE CATT, THE INCITANT OF STEM ROT OF PADDY IN BIHAR

A. P. MISRA, ABU MOHAMMAD AND P. K. DAS

(Accepted for publication Jan. 9, 1966)

The stem rot disease of paddy (*S. oryzae* Catt.) had been serious in recent years in certain localities of Bihar. Misra and Mohammad (1964) investigated the pathogenicity and varietal reaction of *S. oryzae* to certain varieties of paddy at Sabour. Earlier Mundkur (1935), Luthra and Sattar (1936) and Paracer and Luthra (1944) had carried out investigations on the disease in Pusa (Bihar) and the Punjab. No studies were, however, made by these authors on the viability of sclerotia, although such studies were made in Ceylon by Park and Bertus (1932), in Philippines by Silayan (1937) and in U. S. A. by Tullis and Cralley (1941). As there may be differences in the viability of sclerotia of different isolates of the pathogen and their survival may also depend on the local environmental conditions, studies were made on the effect of storage under the soil and environmental conditions existing at Sabour and treatment with chemicals and hot water with an isolate of the pathogen from Bikramgunj area in Shahabad, Bihar.

MATERIALS AND METHODS : The fungus was isolated in pure culture from single sclerotium taken from diseased paddy plants collected from Bikramgunj (Shahabad, Bihar), where the stem rot disease had been severe in certain localities in recent years. Sclerotia collected from one-month old cultures on potato dextrose agar were used in each treatment except when stated otherwise to ensure maximum uniformity of age, conditions of development and purity of isolate. After each treatment, fifty sclerotia were taken out from each replication, surface-sterilised in 0.1 per cent mercuric chloride solution for 1 minute, washed in (3-4) changes of sterile water and then planted on potato dextrose agar and incubated at 30°C. In studies on the effect of chemical treatment, the sclerotia after such treatment were similarly washed and planted on potato dextrose-agar and incubated. For studies on thermal inactivation, the sclerotia were grown on rice stems (Bega and Smith, 1962) and subjected to hot water treatment at different temperatures and later planted on potato dextrose agar and incubated at 30°C.

1. *Effect of storage under laboratory conditions on the viability of sclerotia of S. oryzae* :

Mature sclerotia of *S. oryzae* collected from one month old culture on potato dextrose agar were stored in corked specimen tubes in November 1962 and kept inside the laboratory at room temperature,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Their viability was tested at monthly intervals according to the method described under Materials and Methods. The percentage of germinated sclerotia was recorded. It will be observed from Fig. 1 that almost all sclerotia lost their viability after 18 months of storage inside the laboratory where the maximum and minimum temperatures during the winter months were 26.1°C and 1.7°C and during the summer months were 37.1°C and 19°C respectively. The maximum and minimum humidity during those periods were 95 and 28 and 98 and 43 during 1962-63.

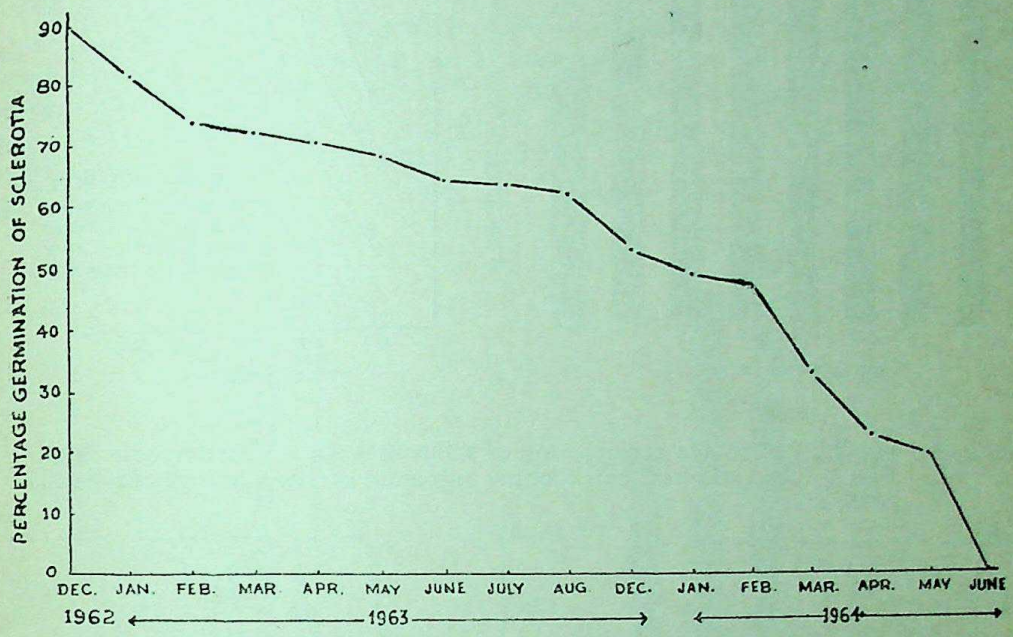


Fig. 1. Effect of storage under laboratory conditions on the viability of sclerotia of *S. oryzae*.

2. *Effect of soil depth on the viability of sclerotia of S. oryzae* :

The viability of sclerotia at different soil depths inside the laboratory was next investigated as follows : Sclerotia collected from one month old culture on potato dextrose agar were placed at the bottom of glass beakers and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 inches of oven-dried soil was placed over them. The beakers were kept inside the incubator at 30°C for a period of two months. The viability of sclerotia was then tested as before. The data have been illustrated in fig. 2. It is evident that higher percentage of sclerotia buried at 0.5 inch soil depth germinated and the germination decreased with increase in soil depth. The viability studies at greater soil depths were not carried out, but it is very likely that their viability may further decrease at greater soil depths.

3. *Study of the effect of chemical and hot water treatment on the viability of sclerotia of S. oryzae* :

(a) *Chemicals* : Sclerotia were treated with copper-sulphate, formaldehyde and mercuric chloride solutions at different concentrations

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

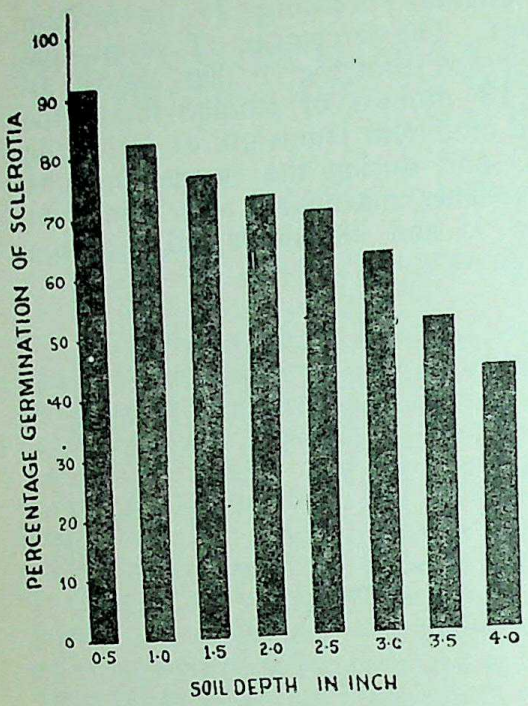


FIG. 2

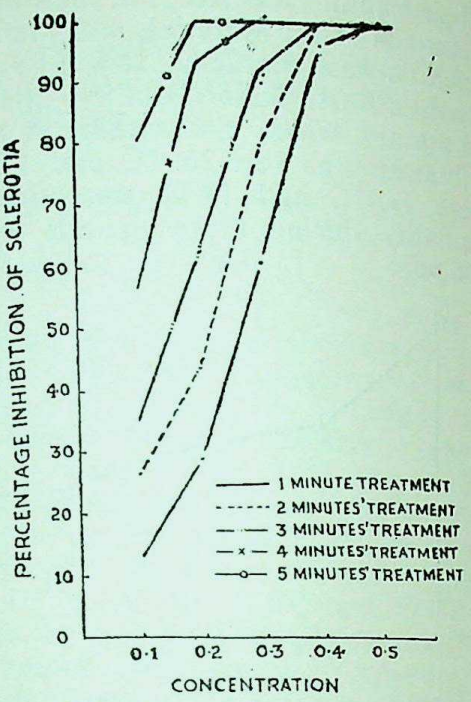


FIG. 3

Figs. 2—3, Fig. 2. Percentage germination of sclerotia kept at different soil depths
Fig. 3. Effect of chemicals (copper sulphate) on the viability of sclerotia of *S. oryzae*.

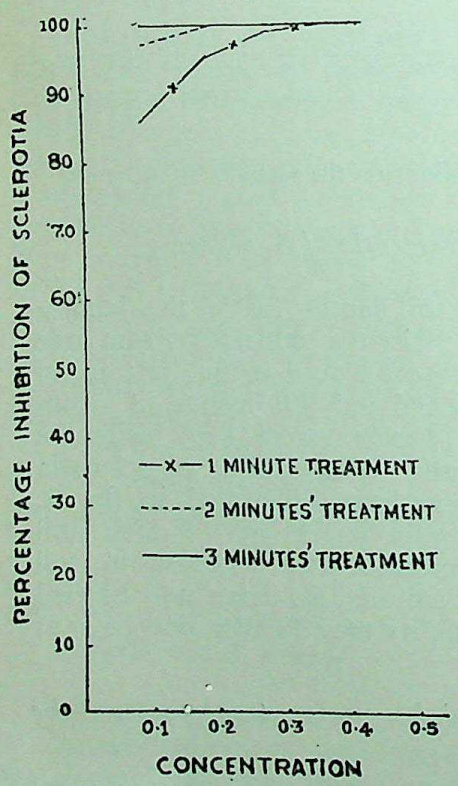


FIG. 4

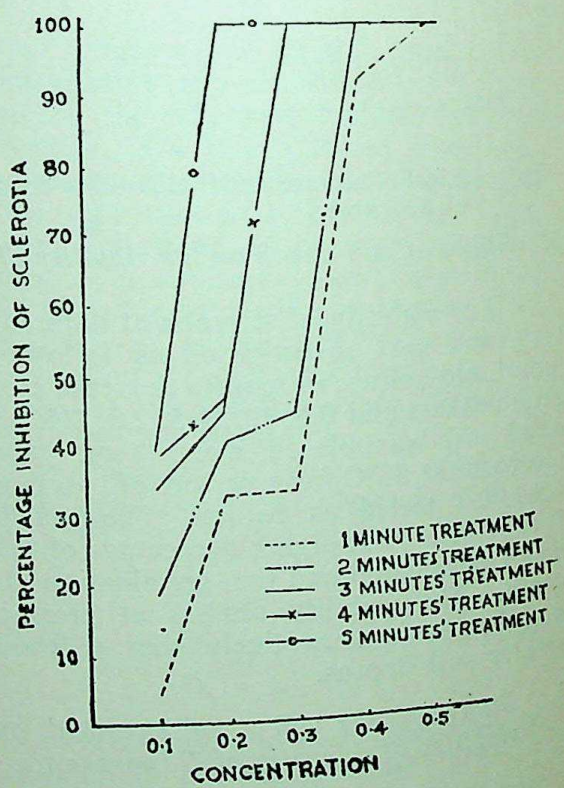


FIG. 5

Fig. 4. Effect of chemicals (formaldehyde) on the viability of sclerotia of *S. oryzae*
Fig. 5. Effect of chemicals (mercuric chloride) on the viability of sclerotia of *S. oryzae*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

for different periods of time and their viability tested as before. The results have been illustrated in Figs. 3, 4 and 5. It was observed that the viability of sclerotia was lost in 1 minute, 2 minutes and 3 minutes respectively at 0.4 per cent, 0.2 per cent and 0.1 per cent concentrations of formaldehyde. In case of mercuric chloride and copper sulphate solutions the complete loss in viability was obtained after 1 minute's treatment at 0.5 per cent or 5 minutes treatment at 0.2 per cent concentration. Formaldehyde thus proved to be most toxic among the three chemicals.

(b) *Hot water* : Sclerotia were colonized on pieces of rice stem and subjected to hot water treatment at different temperatures for 1 minute and their viability tested on potato-dextrose agar at 30°C. The results have been presented in Fig. 6. It is observed that 66 per cent inhibition of sclerotia was found at 70°C and complete inactivation at 80°C.

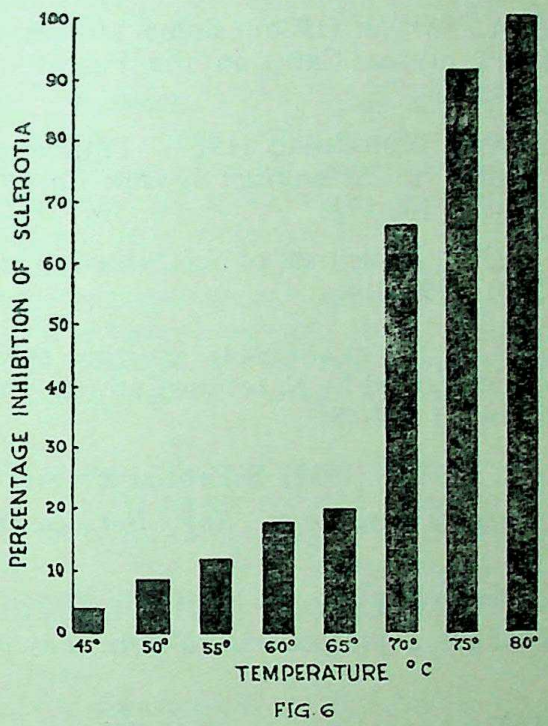


Fig. 6. Effect of hot water treatment on the viability of sclerotia of *S. oryzae*

SUMMARY

Sclerotia of *Sclerotium oryzae* Catt. lost their viability after 18 months storage at room temperature at Sabour. Sclerotia when placed under oven-dried soil at the bottom of glass beakers for two months at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 inches depth inside an incubator at 30°C did not completely lose viability ; 91.6, 82.2, 76.3, 72.6, 69.5, 62.3, 51.2, 43.2 per cent respectively germinated at the end of the period. Of the three chemicals, formaldehyde, copper sulphate and mercuric

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

chloride, formaldehyde was most toxic at 0.4, 0.2 and 0.1 per cent concentration, the sclerotia lost viability in 1, 2 and 3 minutes respectively. The sclerotia were completely inactivated after 1 minute's treatment in hot water at 80°C.

ACKNOWLEDGEMENT : Thanks are due to Sri R. S. Roy, Principal, Bihar Agricultural College, Sabour for granting facilities for this work.

Department of Mycology and Plant Pathology,
Bihar Agricultural College, Sabour,
(Bhagalpur).

REFERENCES

BEGA, R. V. AND R. S. SMITH (1962) Time-temperature relationship in thermal inactivation of sclerotia of *Macrophomina phaseoli*. *Phytopathology* **52** (7) : 632.

LUTHRA, J. C. AND A. SATTAR (1936) Some studies on the sclerotial disease of rice (*S. oryzae* Catt.) in the Punjab. *Indian J. Agric. Sci.* **6** : (4) ; 973-984.

MISRA. A. P. AND ABU MOHAMMAD (1964) Pathogenicity and varietal reaction of paddy to *Sclerotium oryzae* Catt. in Bihar. *Indian Phytopath.* **17** (2) : 168-171.

MUNDKUR, B. B. (1935) Parasitism of *Sclerotium oryzae* Catt. *Indian J. Agric. Sci.* **5** (2) : 393-414.

PARACER, C. S. AND J. C. LUTHRA (1944) Further studies on the stem rot disease of rice caused by *S. oryzae* Catt. in the Punjab. *Indian J. Agric. Sci.* **14** (1) : 44-48.

*PARK, M. AND L. S. BERTUS (1932) Sclerotial diseases of rice in Ceylon. 2. *Sclerotium oryzae* Catt. *Ann. Roy. Bot. Gard., Peradeniya* **11** (4) : 342-359.

*TULLIS, E. C. AND E. M. CRALLEY (1941) Longevity of sclerotia of the stem rot fungus *Leptosphaeria salvinii*. *Phytopathology* **31** (3) : 279-281.

*SILAYAN, H. S. (1937) Annual Report of the Director of Plant Industries Commonwealth of Philippines for the fiscal year ending Dec. 31, 1936.

*Originals not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A MOSAIC DISEASE OF PETUNIA

S. C. MISRA AND V. V. CHENULU

(Accepted for publication December 29, 1965)

INTRODUCTION : *Petunia hybrida* Vilm. grown in flower beds of various Divisions of Indian Agricultural Research Institute has been found frequently affected by a disease characterised by typical mosaic mottling. Petunia mosaic is caused by several viruses viz., tobacco mosaic virus, Alfalfa mosaic virus, Datura mosaic virus, tomato bushy stunt virus, potato virus, cucumber mosaic virus, etc., either under natural conditions or by artificial inoculations. Results of studies carried out to determine the identity of the virus affecting *Petunia* plants at Indian Agricultural Research Institute are reported in this paper.

MATERIAL AND METHODS : The virus was isolated from mosaic infected Petunia plants. The culture was maintained on *Nicotiana tabacum* var. White Burley and sap from infected leaves of *N. tabacum* was used as inoculum unless otherwise stated. The inoculum was prepared from infected leaves of *N. tabacum* showing typical symptoms of the disease and sap inoculation tests were carried out by the usual rubbing method using carborundum powder (600 mesh) as an abrasive. The standard extract used for inoculation in some of the experiments was prepared by adding 1 ml. of distilled water for every gram of leaf tissue used for extraction of sap.

RESULTS : *Symptoms* : Petunia plants inoculated with the virus are stunted in growth. The leaves are reduced in size. The young leaves exhibit clear mosaic mottling comprising of irregular light green and dark green patches (Fig. 1). The lower leaves exhibit the symptom of vein banding. In some cases the leaves are malformed with blisters and raised green areas.

Host range : For determining the host range of the virus, plants belonging to 56 genera and 11 families were inoculated. All the host plants were raised in 4" pots filled with sterilized soil inside insect—proof glass house. The plants were inoculated in 3-4 leaf stage and kept under observation for a period of 6-8 weeks. Plant species that did not show any visible symptoms were indexed on healthy tobacco plants to find out if they were carrying the virus symptomlessly.

The virus was successfully transmitted to a few members of the family Solanaceae viz., *Nicotiana tabacum* L. Cultivars White Burley and Harrison Special, *N. glutinosa* L., *N. rustica* L., *Capsicum annuum* L. and *Petunia hybrida* Vilm. and symptoms produced on these hosts are briefly described.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Nicotiana tabacum L. Cultivars. White Burley and Harrison Special. and *N. rustica* :

The first symptoms on these hosts appear 30-35 days after inoculation in December-January and after only 4-5 days in the summer months. These symptoms appear as yellow spots accompanied by vein clearing. After a week the yellowish spots aggregate to form patches of yellow green areas. The leaves which develop later are very much reduced in size and show mosaic mottling. Occasionally malformation of leaves, green vein banding and blistering of the leaf surface is noticed. In acute cases the leaf lamina is very much reduced in one or both sides of the midrib giving it a rather filiform appearance. (Figs. 3 and 4).

N. glutinosa L. : On the leaf next to the inoculated leaf a number of yellow spots appear all over the surface. The leaf size is very much reduced. The small yellow spots coalesce after sometime forming large chlorotic areas. As the plants grow older light green and dark green areas appear uniformly all over the leaf giving it a mosaic appearance (Fig. 2). With the onset of warmer conditions the symptoms get completely masked.

Capsicum annuum L. : The first symptom is a clear vein clearing of the youngest leaf. This is soon followed by severe mottling of the leaves (Fig. 5). Blistering and puckering of the leaves is never observed. Reduction of leaf size or stunting of growth is not noticed.

The virus did not infect the following species.

Solanaceae : *Solanum nigrum* L., *S. melongena* L., *S. pseudocapsicum* L., *Physalis floridana* Rydb., *P. peruviana* L., *Lycopersicon esculentum* L., *L. esculentum* var. Pusa Ruby, *Datura stramonium* var. Fatula (E. C. 24818) *D. stramonium* (E. C. 15656), *D. metaloides* DC (E. C 25086), *D. fastuosa* (E. C. 26029), *D. arnecifolia* L. (E. C. 25808), *D. metal* L. (E. C. 25976), *D. inoxia* L. (E. C. 1195), *D. ferox* L. (E. C. 1194), *D. bernhardii* Lindstrom (E. C. 19706).

Compositae : *Zinnia elegans* Jacq.

Cucubitaceae : *Cucumis sativus* L. (I. C. 10665), *C. melo* L. (I. C. 10285), *Cucurbita pepo* L. var. Corogated, *C. pepo* L. var. Oblong, *C. maxima* L., *Citrulus vulgaris* Schrad., *Lagenaria leucantha* (Duch.) Rushy., *Luffa acutangula* Roxb. var. Pusa Chickni, *L. acutangula* (I. C. 8812), *Trichosanthus anguina* L.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

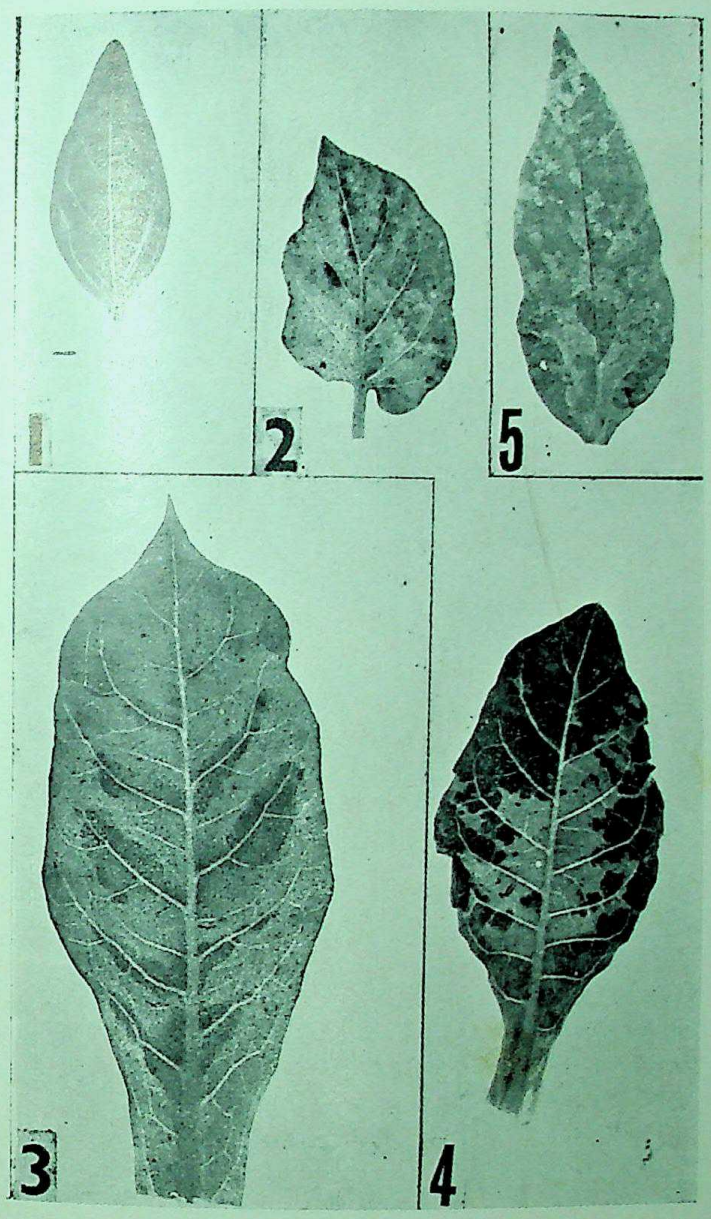


Fig. 1—5. Symptoms caused by the virus from mosaic infected petunia plants on (1) *Petunia hybrida* (2) *Nicotiana glutinosa* (3) *Nicotiana tabacum* var. Harrison Special (4) *N. tabacum* var. White Burley and (5) *Capsicum annuum*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Chenopodiaceae : *Beta vulgaris* L. var. Polyx. *B. vulgaris* L. var. D.D.R., *Spinacea oleracea* L., *Chenopodium amaranticolor* Coste and Reyn. *C. murale* L., *C. album* L., *C. nuttaliae* Satt. *C. quinoa* Willd.

Leguminaceae : *Vigna sinensis* (Torner) Savi. *Lupinus angustifolius* L. (I. C. 6434), *Phaseolus vulgaris* L., *Vicia faba* L., *Pisum sativum* L.

Cruciferae : *Brassica oleracea* L.var. capitata, *B. oleracea* L. var. botrytis., *B. oleracea* L. var. Sarson Prain. Umcelliferae : *Dacus carota* L. var Asiaticred ; Polygonaceae : *Fagopyrum esculentum* Moench (I. C. 7915). Phytolaccaceae : *Phytolacca ameriana* L., Amarantaceae : *Amaratnhus caudatus* L., *A. cruentus* L., *A. gangeticus* L. *Gomphrena globosa* L.

TRANSMISSION OF THE DISEASE : The virus is transmitted mechanically and the incubation period varied depending on the temperature. During December-February the incubation period in different susceptible hosts ranged from 30-35 days, while in summer months with temperatures above 90°F the incubation period was reduced to 4-5 days. The symptoms in different hosts are more pronounced at low temperatures than at high temperatures.

The virus is also transmitted by *Aphis gossypii* Glov. Three other aphids viz., *Aphis crassivora* Koch., *Myzus persicae* Sulz. and *Lipaphis erysimi* Kaltenbeck, failed to transmit the disease.

PROPERTIES OF THE VIRUS : The virus in standard extract tolerated exposure to 58°C for 10 minutes but was inactivated at 60°C. The virus in sap retained infectivity at 1 : 100 dilution but not at 1 : 200. It lost infectivity between 92-96 hours storage at room temperature (25-35°C) and 96-108 hours at 7°C. The virus inside infected leaves of *N. tabacum* withstood desiccation for 4 days but not for 5 days at room temperature (25-35°C). Freezing virus infected leaves for 7 days resulted in loss of infection. Chemicals such as saffranine (0.4 per cent), lead sub acetate (1 per cent) ethyl alcohol (48 per cent), did not affect the infectivity of the virus when mixed in equal volumes with clarified sap. Thiouracil (200 ppm) and 8-azaguanine (200 ppm) however, showed inhibition of the virus *in vitro* to the extent of 16-33 per cent.

DISCUSSION : A large number of viruses has been reported to infect and cause mosaic symptoms on Petunia either under experimental or natural conditions. Chief among the viruses reported to cause mosaic symptoms on Petunia are Petunia mosaic virus (Bewley 1923 ; Smith 1929 ; Ghimpu 1933 ; Matsumoto and Hirane 1939 ; Yu 1939 ; Eristavi 1941 ; Jones 1949 ; Rubio and Rosell 1959 and Rubio 1959); Datura mosaic virus (Anonymous 1943 ; Capoor and Varma 1948 ; Garga 1948) ; Alfalfa mosaic virus (Pierce 1934 ; Zaumeyer 1938) ; Tomato bushy stunt virus (Lovisolo 1956) ; Potato virus y (Debakx 1961; Smith 1931 ; Dykstra 1933 ; Vasudeva and Lal 1945 ; Braeak 1960) ;

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Tobacco mosaic virus (Allard 1916; Johnson 1926); Cucumber mosaic virus (Johnson 1926 ; Wellman 1935 ; Chamberlain 1939) ; Lucerne mosaic virus (Berkeley 1947) ; Chilli mosaic virus (Dale 1954 ; Jha and Raychaudhuri 1956 ; Mishra 1963) and Chrysanthemum mosaic virus (Kristensen, 1954).

A comparison of the host range and properties of the virus studied with the several viruses reported in literature to cause mosaic of Petunia has revealed that its properties do not resemble entirely with any one of them. The virus infecting Petunia at Delhi differs distinctly from viruses such as tobacco mosaic virus and tomato bushy stunt viruses both in its limited host range as also in physical properties. Also several such as lucerne and chrysanthemum mosaic viruses do not have any resemblance to the virus in respect of physical properties, insect vector and host range. The virus differs from potato virus Y (PVY) in its inabillity to infect *Physalis floridana* the differential host of PVY and also by its failure to be transmitted by *Myzus persicae* the vector of PVY.

The virus studied, resembles Chilli mosaic virus of Dale (1954) ; and of Jha and Raychaudhuri (1956) in thermal death point and transmissibility by *Aphis gossypii*. But it differs from ChMV in other properties such as dilution end point, lengevity *in vitro* and host range. The Chilli mosaic virus reported by Jha and Raychaudhuri (1956) has a wider host range affecting members of Solanceae, Cucurbitaceae, and Compositae. The host range of the present virus is restricted to a few members of the family Solanaceae. The Chilli mosaic virus of Mishra (1963) differs from this virus widely both in host range as well as physical properties. The virus has very close resemblance to Petunia yellow mottle of Rubio and Rosell (1959) in properties such as thermal death point, dilution end point, but differs in its reaction on *N. tabacum*. The virus reported herein causes systemic mottle on *N. tabacum* while Petunia yellow mottle virus causes necrotic local lesions. A strict comparison of the virus with Petunia mosaic virus of Bewley (1923) is not possible as Bewley did not study the properties. Because of its close resemblance in host range and properties to Chilli mosaic virus of Jha and Raychaudhuri (1956) and Petnuia yellow mottle of Rubio and Rosell (1959), the Delhi isolate responsible for mosaic of Petunia has been regarded as a strain of either of these viruses. Establishment of strain relationship with Petunia yellow mottle of Rubio and Rosell (1959) or Chilli mosaic of Jha and Raychaudhry (1956) by conventional methods of cross protection or serology is not possible as cultures of both the viruses are not available

SUMMARY

Symptoms, host range and properties of a virus causing mosaic of Petunia plants has been studied The disease is characterised by conspicuous mosaic mottling of the infected leaves, stunting and smalling of the foliage. The virus is transmitted mechanically by sap inoculation. It is also transmitted by the aphid vector *Aphis gossypii*. The host range of the virus is restricted to a few members of the family Solanaceae viz.,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Nicotiana tabacum cultivars. White Burley and Harrison Special, *N. glutinosa*, *N. rustica* and *Capsicum annuum*. The virus failed to infect 50 other host species belonging to 10 families. The incubation period of the virus in *N. tabacum* was 30-35 days in winter and 4-5 days in summer. The thermal death point of the virus was 60°C, the dilution end point was 1 : 200 and longevity *in vitro* was 96 hours at room temperature (25-30°C) and 108 hours at 7°C. The virus in infected leaves withstood freezing for 144 hours and desiccation for 96 hours at room temperature (25-30°C). Of the various chemicals tested for the inhibition of the virus only Thiouracil and 8-azaguanine at 200 ppm reduced infectivity of the virus *in vitro*. A comparison of the virus with several other viruses reported to cause mosaic of Petunia either naturally or under artificial conditions has shown that the virus is a strain of either Chilli mosaic virus of Jha and Raychaudhuri (1956) or Petunia yellow mottle of Rubio and Rosell (1959).

ACKNOWLEDGEMENTS : We are grateful to Dr. B.L. Chona former head of the Division of Mycology and Plant Pathology, for providing necessary facilities and encouragement. It is with great pleasure we acknowledge our grateful thanks to Dr. S. P. Raychaudhuri, Head of the Division of Mycology, for critically going through the manuscript and helpful suggestions. The senior author is especially grateful to Director, Indian Agricultural Research Institute for the award of I. A. R. I. fellowship, during the course of these investigations.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

REFERENCES

ALLARD H. A. (1916) The mosaic diseases of tomatoes and petunia. *Phytopathology* **6** : 328-335.

ANONYMOUS. (1943) Agricultural Research. *Imperial Council Agric. Res.* Delhi, 1941-42 : 3-39.

BERKELEY, G. H. (1947) Alfalfa mosaic on pepper in Ontario (Abstract). *Phytopathology* **37** : 3.

BEWLEY, W. F. (1923) Mycological report. *Eighth Ann. Rep. Chestnut Exp.* and *Res. Sta. Hertfordshire 1922* : 34-35.

BRAEAK, J. (1960) On the use of potato hybrids for quick determination of virus and on the possibillity of occurrence of tobacco virus browning in Czechoslavakia. (Abs.) *Preslia* **32** : 398-422.

CAPOOR, S. P. AND P. M. VARMA (1948) Mosaic of *Datura alba*. *Curr. Sci.* **17** : 151.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHAMBERLAIN, E. E. (1939) Cucumber mosaic *N. Z. J. Sci. Tech.* **21** : 79-80.

DALE, W. T. (1954) Sap transmissible mosaic diseases of Solaneceous crops in Trinidad. *Ann. appl. Biol.* **4** : 240-247.

DEBAKX J. A. (1961) Waard planten van het A ardappel y^n virus. *J. Zjdsclair Plziekt.* **67** : 273-277 (English summary).

DYKSTRA, T. P. (1933) Weeds as possible carriers of leaf roll and rugose mosaic of potato. *J. agric. Res.* **157** : 17-32.

ERISTAVI, E. M. (1941) Plant virus diseases and their control. *Moscow Leningrad Acad. Sci. U. S. S. R.* **11** : 4-7.

GARGA, R. P. (1958) Studies on virus diseases of plants in M.P. II. A new strain of Datura virus 3. *Indian Phytopath.* **11** : 133-136.

GHIMPU, V. (1933) Surles maladies a virus de quelques Solanaceous. *Competes rendus Soc. de Biol.* **112** : 1113-1115 (R. A. M. **12** : 508,1933).

JHA, A. AND S.P. RAYCHAUDHURI (1956) Mosaic disease of chilli (*Capsicum frutescence*). *Indian J. Agric. Sci.* **26** : 217-222.

JOHNSON, J. (1926) Mosaic diseases on differential hosts. *Phytopathology* **16** : 141-149.

JONES, L. H. (1949) Department of Botany. *Rep, Mass. Agric. Exp. Sta.* 1948-1949 (Bull. 453), 19-26.

KRISTENSEN, H. R. (1954) Chrysanthemum virus Yg dommi Arrsskr. *Dansk. chrysanth. Selsk.* 31-37.

*LOVISOLO, O. (1956) Petunia nuovo ospits natural delvins del rachitismo cepriglioso del Pomodro. *Boll. Staz. Pat. Veg. Roma Ser.* 3-14 : 103-199.

MATSUMOTO, T. AND S. HIRANE (1939) Two strains of Petunia mosaic. *Trans. Nat. Hist. Soc. Formosa* **29** : 184-185.

MISHRA, M. D. (1963) Host range and physical properties of a virus causing mosaic symptoms and necrosis on Chillies (*Capsicum annuum*). *Indian J. Microbiol* **111** : 77-84.

*RUBIO, M. (1959) Nota Previa sobre un virus de tipo 'ringspot' encontrado en *Petunia hybrida. Microbiol esp.* **12** : 325-330.

*RUBIO M. AND M. ROSELL, (1959) Estudio de un neuvo rinus encontrado en *Petunia hybrida. Microbiol esp.* **12** : 105-138.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SMITH K. M. (1929) Studies on potato virus diseases IV. Further experiments with potato mosaic. *Ann. appl. Biol.* **16** : 1-33.

SMITH, K. M. (1931) On the composite nature of certain viruses. *Proc. Roy. Soc.* Ser. B. **109** : 251-267.

VASUDEVA, R. S. AND T. B. LALL (1945) Studies on the virus diseases of potatoes in India. II. *Solanium virus* 2. *Indian J. agric. Sci.* **15** : 240-242.

WELLMAN F. L. (1935) The host range of southern celery mosaic virus. *Phytopathology* **25** : 376-404.

YU, T. T. (1939) A list of plant viruses observed in China. *Phytopathology* **29** : 459-461.

ZAUMEYER W. J. (1939) A streak disease of peas and its relation to several strains of Alfalfa mosaic virus. *J. agric. Res.* **56** : 747-772.

* Original not seen

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

NEMATOCIDAL PROPERTIES OF ANAGALLIS ARVENSIS L. EXTRACTS

Y. L. NENE AND P. N. THAPLIYAL

(Accepted for publication December 21, 1965)

INTRODUCTION : Recently the authors (Nene and Thapliyal, 1965) reported the discovery of antifungal properties of the extracts of *Anagallis arvensis* L. which is a weed very commonly found in north India during winters and also grows in different parts of the world. In India, the weed has been used for repelling leeches from the nostrils of farm animals and for medical purposes (Anon. 1948). So far there are no reports on the nematocidal properties of the weed. The present paper reports the findings concerning its nematocidal properties.

MATERIAL AND METHODS : The extracts were prepared from the dried naturally-found shoots which were collected before the fruiting stage during January and February, 1965. One gram of fresh plant material produced 0·16 gm. of dried material and thus extracts, unless otherwise mentioned, were prepared by grinding 0·16 gm. dried plant material per ml. of sterilized distilled water and filtering through Whatman No. 1 filter paper.

Larvae of the wheat ear-cockle nematode, *Anguina tritici* (Steinbuch) Filipjev obtained from the galls were used for the tests. The procedure to obtain larvae for tests consisted in soaking an average size gall (3×2mm.) in 20 ml. of sterilized distilled water for two hours, slightly pressing it with a glass rod to release larvae in water, and allowing them about 6 hours to become active. Such a suspension was diluted five times with water before use in experiments. One drop of this suspension from 10 ml. pippette normally contained 10 to 15 larvae. The tests were conducted in glass tubes with a height of 75 mm. and an internal diameter of 21 mm. All the experiments were carried out at least twice.

RESULTS : *Ascertaining adverse effect* : The treatment-tubes containing 5 ml. of 1 : 1 filtered *Anagallis* extract and two drops of the nematode suspension, having about 20-30 larvae, were transferred to these with a ten ml. pippette. The control-tubes contained two drops of nematode suspension in 5 ml. of water. The observations were made six hours after the start of the experiment by mounting three drops with a ten ml. pippette on a glass slide and observing under a microscope. All the contents in the tubes were also examined to observe the effect on all the larvae by pouring the contents in petri dishes and observing under a low-power microscope.

Six hours after the start of the experiment, the larvae in treatment-tubes were found to be lying straight without any motion, whereas larvae from control-tubes were active and moving characteristically.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Whether the effect is nematostatic or nematocidal ? An experiment was conducted to determine whether the adverse effect of *Anagallis* extract was nematostatic or nematocidal. The larvae were treated with *Anagallis* extract for six hours as described in the preceding experiment and then transferred with the help of a fine needle to a petri dish cover containing sterilized distilled water. This was done to remove the traces of *Anagallis* extract from the surface of the larvae. Two more transfers were made and these larvae were observed every six hours upto 24 hours.

The larvae failed to regain their ability to move, indicating the effect of *Anagallis* extract to be nematocidal.

Effective dilution : Attempts were made to find out the extent of dilution to which the effectiveness of the *Anagallis* extracts could be observed. The original extract was prepared as described under methods. This was called 1 : 1 extract. Further dilutions viz. 1 : 5, 1 : 10, and 1 : 100 were obtained by adding requisite quantities of sterile water. The nematode larvae were introduced into tubes containing these diluted extracts and the effect was observed after six hours.

It can be seen from the data in Table 1 that all larvae died in six hours when kept in contact with extracts diluted upto 1 : 10, whereas with 1 : 50 and 1 : 100 extracts the nematocidal effect gradually diminished. A dilution of 1 : 1000 was tried later and was found ineffective in killing the larvae.

Thermostability : (a) *Boiling at room temperature* : An experiment was conducted to find out the ability of the nematocidal principle to withstand heat. Extracts diluted 1 : 10 were used in these tests. The treatments consisted of boiling the extracts, for 1, 3, and 5 minutes. The larvae were introduced into the tubes containing these boiled extracts. The control-tubes cotained boiled water instead of extracts. Observations were made after six hours.

All the nematode larvae under each treatment were killed within six hours indicating thereby the retention of the nematocidal property by the extracts after boiling.

(b) *Autoclaving* : Extract was autoclaved at 15 lb. pressure for 25 minutes before using for tests. The tests were carried cut as in the last experiment. The nematocidal activity was evident in the extract as all the nematodes were found dead in six hours.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Nematocidal activity of *Anagallis arvensis* L. extracts at various dilutions.

Dilution	Average No. of larvae[1]		Percentage	
	Living	Dead	Living	Dead
1 : 1	0.0	23.3	0.0	100.0
1 : 5	0.0	22.0	0.0	100.0
1 : 10	0.0	22.6	0.0	100.0
1 : 50	4.0	20.6	16.2	83·8
1 : 100	8.7	16.3	34.8	65.2

1. Average of two trials with four replications in each.

Ageing : In order to find out whether the nematocidal substance in extracts withstands ageing at room temperature, an experiment was conducted in which *Anagallis* extract (1 : 10) was kept at room temperature (30–34°C) for seven days in corked tubes. This extract was used on the eighth day in the tests. The nematocidal property was retained as all the nematode larvae were found dead when observed six hours after the start of the experiment.

Influence of pH : In order to find out whether the nematocidal property is exhibited at different pH or not, an experiment was conducted in which pH of the extract was varied from 3.5 to 9.5 by adding 1 N HCL or 6 N NaOH. The extracts used for varying pH were of 1 : 10 dilution and had the original pH of about 6.0. These extracts at different pH were kept at room temperature for about an hour for a final check-up of pH before using for tests. All the nematode larvae at all the different pH were found dead six hours after the start of the experiment indicating no influence of pH on the nematocidal property of the extract.

DISCUSSION : Strong nematocidal properties are exhibited by the extracts of *Anagallis arvensis* L. *Anagallis* extract is known to be leech repellent, dog poison, fish poison (Anon., 1948), antifungal (Nene and Thapliyal, 1965), and insecticidal (Frear, 1948). The present report is the first about its nematocidal properties.

The nematocidal principle appears to be quite stable, since influence of heat, ageing, and pH did not alter the nematocidal properties. Thus the isolation and purification of the nematocidal principle should not be a difficult problem.

The amount of nematocidal principle in the extracts does not appear to be substantial since dilution of 1 : 100 was only partially effective and the dilution of 1 : 1000 was ineffective. This is in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

contrast with the amounts of antifungal principle present in these extracts because a dilution of 1 : 200,000 was reported to be effective against the test fungus, *Colletotrichum papayae* (P. Henn) P. Henn. by the authors (Nene and Thapliyal, 1965).

According to Wehmer (1931), *Anagallis arvensis* extracts contain two glucosidic saponins. The work is in progress to determine whether these or some other compounds are responsible for the nematocidal properties.

SUMMARY

Extracts of *Anagallis arvensis* L. show nematocidal properties. The nematocidal principle appears to be quite stable as it withstands influence of heat, ageing and pH. Extracts diluted to 1 : 10 with water are strongly nematocidal, but this effect diminishes with further dilutions. Attempts to isolate the active principle are in progress.

ACKNOWLEDGEMENTS : Thanks are due to Dr. R.L. Paliwal, Director of Experiment Station, for providing facilities and to Dr. N.K. Anant Rao, Dean, College of Agriculture and Dr. R.S. Singh, Asstt. Professor of Plant Pathology, for going through the manuscript.

Department of Plant Pathology,
U.P. Agricultural University,
Pantnagar, Distt. Naini Tal.
U.P.

REFERENCES

ANONYMOUS (1948) *The Wealth of India*. Council of Scientific and Industrial Research. Raw Materials 1 : 75.

FREAR, D.E.H. (1948) *Chemical fungicides and plant insecticides*. Vol. II. Chronica Botanica Co., Waltham, Mass.

NENE, Y.L. AND P.N. THAPLIYAL (1965) Antifungal properties of *Anagallis arvensis* L. extract. *Naturwissenschaften* **52** : 89-90.

*WEHMER, C. (1931) Die Pflanzenstoffe 2 : 923.

*Original not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE EFFECT OF CERTAIN NUTRIENTS ON THE CONIDIAL GERMINATION IN COLLETOTRICHUM FALCATUM

PRITAM SINGH[1]

(Accepted for publication November 12, 1965)

Fungal spores are known to differ considerably in their nutritional requirements for germination (Gottlieb, 1950 ; Lilly and Barnett, 1951 ; Cochrane, 1958). In *Colletotrichum falcatum* Went, the causal organism of the red rot of sugarcane, considerable work has been done on the requirement of various carbon and nitrogen sources, vitamins and hormones, during the growth of the fungus (Ramakrishnan, 1941 ; Chatrath and Bajaj, 1964), but nothing seems to be on record regarding their role in the germination of conidia. In view of poor germination of the conidia of *C. falcatum* in water (14-18 per cent in the light type isolates and 36-40 per cent in the dark type ones (Singh, 1962), it was considered desirable to investigate into the possibility of improving their germination by the addition of some nutrients to the slide drop. This paper records the effect of different carbon and nitrogen sources, vitamins and plant growth regulators on the germination of conidia of *C. falcatum.*

MATERIAL AND METHODS : Actively growing (12-16 days old) single spore cultures of three light type, virulent, highly sporing isolates (Nos.* 244, 301 and strain 'I') and three dark type, weakly pathogenic and sparsely sporing ones (Nos.* 7, 78 and 300) were employed. The cultures were maintained on Oat-meal agar medium.

Solutions of different carbohydrates [d(+) xylose, l (+) rhamnose, fructose, glucose, galactose, sucrose, lactose, raffinose, starch and glycogen] were prepared in such concentrations in distilled water so that the total amount of carbon made available was equivalent to the amount of carbon present in 3 per cent sucrose.

Potassium nitrate (KNo_3) potassium nitrite (KNo_2), sodium nitrate ($NaNo_3$) and ammonium nitrate (NH_4No_3) were selected as sources of inorganic nitrogen, while casein and peptone were used as organic nitrogen sources. Glycine, dl-alanine, dl-valine, l-leucine, dl-serine, dl-threonine, dl-aspartic acid, l-glutamic acid, l-arginine, dl-phenyl alanine and asparagine were used as sources of amino nitrogen. Solutions of the different nitrogen sources (inorganic, organic and amino acids) were prepared in such concentrations so that the nitrogen made available was equivalent to the amount of nitrogen contained in 0.5 per cent asparagine or 0.67 per cent potassium nitrate.

---

*Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi.

[1]Present address : Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin (U. S. A.)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Stock solutions of nine vitamins were prepared in plain distilled water in the following concentrations and stored in acid cleaned 'Pyrex' flasks (100 ml. capacity) at 5°C.

Thiamine	1 mg/litre
Biotin	1 ug/litre
Inositol	200 ug/litre
Pyridoxine	500 ug/litre
Nicotinic acid	500 ug/litre
Pantothenic acid	200 ug/litre
Riboflavin	1 mg/litre
Para-aminobenzoic acid (PABA)	5 mg/litre
Choline	5 mg/litre

Combined effect of a carbon source and a vitamin was studied by supplementing sucrose with seven different vitamins such as thiamine, biotin, riboflavin, choline, PABA, pantothenic acid and inositol. Stock solutions of these vitamins were added to 1.5 per cent sucrose solution in the ratio of 1 : 15.

Alpha-naphthalene acetic acid (NAA), beta-indol butyric acid (IBA), indol acetic acid (IAA), para-chlorophenoxy acetic acid (PCPA) gibberellic acid (GA) and 2, 4-dichlorophenoxy acetic acid (2, 4-D) were the plant growth regulators used in the concentration of 5 ppm.

The various solutions of carbon and nitrogen compounds, vitamins, vitamins+sucrose, amino acids and plant growth regulators were autoclaved, separately, at 10 lbs. pressure for 10 minutes. The pH was adjusted to 6.0 by M/30 phosphate buffer.

The germination for each isolate was studied with a standard conidial* suspension (15-20 spores per microscopic field under the low power of the microscope), made in the different nutrient solutions, in moist chambers, made out of pairs of sterilized Petri plates at 27°C** after an incubation of 18 hours**. Germination in distilled water served as a control. The criterion for the germination of a conidium was the emergence of germ tube to a length exceeding its width. Observations were recorded both on the percentage of germination and the length of the germ tube. The average data for each experiment are based on about 300 spore counts/germ tube length measurements.

RESULTS : *Effect of carbon sources* : Experiments on the effect of different carbon sources on the germination of conidia of different isolates of *C. falcatum* showed that sucrose is the best carbon source for the

*The harvested conidia were freed off adhering external nutrients and mycelial fragments through washing them twice in sterile distilled water by centrifugation (2800 rpm) for 5 minutes, each.

**Optimum temperature and incubation period (Singh, 1962).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

germination of the light type isolates, followed by xylose, fructose, lactose, glucose, starch, galactose, rhamnose, raffinose and glycogen. On the other hand, starch appeared better for the dark type ones, followed by lactose, sucrose, fructose, raffinose, glucose, xylose and galactose. Although dark type isolates germinate best in starch, surprisingly, no germination was noticed in animal starch (glycogen). The longest germ tube was more than 300μ. Appressorial formation was indefinite and the germination was mostly bipolar.

Effect of vitamins : Results of studies with vitamins show that most of them are stimulatory in that they increased the percentage of germination but not the germ tube length. Thiamine induced greatest stimulation of the light type isolates, followed by riboflavin, choline, inositol and PABA. Pantothenic acid and biotin exhibited no effect upon conidial germination, while nicotinic acid and pyridoxine were inhibitory. On the other hand thiamine and riboflavin had the greatest effect on the dark type ones, followed by biotin, PABA and choline. Pantothenic acid had no effect ; while nicotinic acid, pyridoxine and inositol were inhibitory. Appressorial formation was abundant in all cases and the germination was mostly bipolar.

Effect of sucrose when supplemented with vitamins : In earlier experiments, sucrose has been found to be a very good form of utilizable carbon and also some vitamins, both to the light and dark type isolates. In this experiment sucrose was supplemented, separately, with seven different vitamins. The effect was found to be greater when the two were used together than when used alone. Sucrose+thiamine induced maximum germination. The germ tubes were always more than 300μ. Appressorial formation was conspicuous by its absence in most of the isolates. The germination was mostly bipolar.

Effect of nitrogen sources : A study of different nitrogen sources (potassium nitrate, potassium nitrite, sodium nitrate, ammonium nitrate, casein, peptone and amino acids) has revealed that potassium nitrate among the inorganic sources, l-arginine, dl-asparatic acid, dl-valine, casein and peptone among the organic ones, stimulated the germination of all the six isolates. Sodium nitrate was considerably inhibitory, while ammonium nitrate and potassium nitrate were completely inhibitory. On the whole, the organic sources were better as compared to the inorganic ones. The effect was more pronounced on the germination percentage than on the length of the germ tube in case of casein and peptone. However, in case of amino acids, it is the germ tube that was stimulated.

Although some amino acids, stimulated germination, Dl-serine, dl-threonine and glycine inhibited the germination of light type isolates, and dl-threonine, dl-serine and dl-alanine of dark type ones. Unipolar germination was characteristic and appressoria were noticed in many cases.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Effect of plant growth regulators : The effect of plant growth regulators, such as alpha-naphthalene acetic acid, beta-indole butyric acid, indol acetic acid, para-chlorophenoxy acetic acid, gibberellic acid and 2,4-dichlorophenoxy acetic acid, on the germination of conidia of *C. falcatum* was studied and the results presented in Table 1.

TABLE 1 : Effect of plant growth regulators on the conidial germination of *C. falcatum* isolates.

C. falcatum isolate No.	Average germination percentage						
	NAA	IAA	IBA	GA	2, 4-D	PCPA	Control
244	15.2	13.8	19.9	17.8	0	12.8	12.4
357	18.3	15.2	20.0	17.4	0	15.0	14.6
'I'	30.0	28.0	36.4	32.0	0	28.4	26.5
390	22.7	18.5	26.0	21.9	0	17.0	16.2
301	22.1	19.8	27.0	23.6	0	19.6	18.7
304	26.9	22.9	32.4	25.3	0	22.8	22.0
382	11.9	10.2	14.4	13.6	0	9.8	7.9
78	44.8	44.6	52.5	48.0	0	42.1	40.4
300	47.6	48.0	53.7	54.5	0	46.0	45.7
7	42.2	43.3	50.2	50.9	0	39.9	38.9

The results show that most of the hormones do not have any marked influence on germination. But all the six isolates were inhibited by 2, 4-D. Slight stimulatory effect was noticeable in case of IBA and GA, the effect being slightly more on the length of the germ tube. Appressorial formation and unipolar germination was a characteristic feature in all cases.

DISCUSSION : The present investigations record a differential response of the light and the dark type isolates to the various carbon sources. The light type highly virulent isolates show the best response with sucrose, the dark type weakly pathogenic ones with starch. Strangely enough, the dark type isolates did not germinate at all in glycogen. The effective utilization of sucrose by the conidia of light type isolates may be due to the elaboration of sucrose (Singh, 1964). However, the utilization of starch by the dark type ones can be attributed to greater amylase production (Singh, 1965).

Xylose, after autoclaving, was found to be superior to glucose. Emerson (1948) assumed it to be partly due to xylose itself and partly due to furfural formed by slight conversion of xylose on autoclaving. The stimulatory effect of furfural has already been reported elsewhere (Singh, 1965).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Considerable work has been done on the vitamin requirements for the growth of *C. falcatum*. However, nothing seems to be on record regarding the role of these factors in the germination of conidia. Most of the growth factors of B-complex group are stimulatory for the initiation of the germ tube. The light and the dark type isolates are best supported by thiamine and riboflavin. The present findings agree with Cooper (1939), who reported that riboflavin and few other vitamins exhibited positive response of *Colletotrichum gloeosporoides*. Inositol was completely inhibitory to the light type isolates and not so to the dark type ones.

A carbon source (sucrose) with certain vitamins showed greater stimulation than when either of the two is used. Sucrose when supplemented with thiamine is the best stimulator. The mechanism of this synergistic effect, however, is not known so far.

Organic nitrogen (excluding amino acids) from casein, followed by peptone, seems to be better for the germination of conidia of *C. falcatum* as compared to the inorganic ones. The inorganic sources, nitrate nitrogen is better than nitrite, which is completely inhibitory. The inhibitory effect of sodium nitrate can be attributed to the toxic effect of Na ion, as potassium nitrate has been found to be beneficial. However, Cochrane (1965)* suspects that sodium is replacing potassium in some reaction for which it is not suitable. May be there is a competition between the two ions for some sensitive site at which only potassium is active. Complete inhibition by ammonium nitrate is assumed to be due to sudden lowering of pH, as reviewed by Cochrane (1958). The data on utilization of nitrate nitrogen agree with those of Ramakrishnan (1941), who reported nitrate nitrite nitrogen as favourable and unfavourable to the growth of and *C. falcatum*. The latter seems to be due to the toxicity of NO_2 ion (Ohtsuki, 1936 ; Herrick, 1940 ; Grewal, 1955 ; and Tandon and Grewal, 1956).

The amino acids do not have any conspicuous stimulatory effect on the percentage of germination. Some isolates, however, did show response in the growth of the germ tube. All the amino acids are not being equally utilized by the light and the dark type isolates is evident from the varied response.

The diverse behaviour of these isolates to different nitrogen sources, including amino acids, possibly indicate the deficiency of that particular nutrient component in spores or their inability to synthesize these during germination (Ryan, 1948-1949 ; Lilly and Barnett, 1951 ; and Fuchs and Gaertner, 1958). As indicated by Lin (1945), the specific requirements for germination of different fungi could explain the diverse behaviour of the isolates in the present investigations.

The inhibitory effect of certain amino acids, like dl-threonine, dl-serine, glycine and dl-alanine, may perhaps be utilized for controlling the spread of the fungus in or on the host. However, this needs further investigation.

*Personal communication.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

None of the hormones, tried, except 2,4-dichlorophenoxy acetic acid, showed any marked effect, 2, 4-D being completely inhibitory. Indol butyric acid and gibberellic acid, however, did show some increase in the length of the germ tube. Such observations are supported by Lin (1945), Naito and Tani (1951) and Peturson (1951) in several fungi. The inhibitory effect of 2, 4-D could be investigated further as foliar spray for preventing secondary infection of the leaves in view of the work of Davis and Dimond (1956) who induced resistance in tomato shoots to *Fusarium* wilt with the foliage spray of 2, 4-D, L-NAA, and 3-IAA.

In conclusion it can be said that there is a conspicuous differential response of the conidia of different isolates of *C. falcatum* to an external supply of nutrients. In general, the response of the light type isolates to all these nutrients is observed to be uniformly more. Further investigations on this differential response of the light and the dark type isolates might give us some idea for explaining the greater virulence of the light type isolates in view of their ability for better utilization of nutrients in the host.

Summary

The effect of the exogenous supply of nutrients such as carbon and nitrogen sources, vitamins and hormones on the germination of conidia of *C. falcatum* isolates has been studied. Most of the carbohydrates were stimulatory, with the best response of light type isolates in sucrose and that of the dark type ones in starch. Surprisingly, the dark type isolates did not germinate at all in glycogen. Vitamins, on the whole, were highly stimulatory and the response of the isolates seemed to be identical. Comparatively, thiamine and riboflavin were the most utilizable growth factors. A synergistic effeet has been observed when sucrose was supplemented with certain growth factors, like thiamine, riboflavin, etc. Organic sources, such as casein and peptone, served as better sources of nitrogen than the inorganic ones, like potassium nitrate, for the germination of conidia. Ammonium nitrate and potassium nitrite were completely inhibitory, while sodium niträte was partially inhibitory. Amino acids, in general influenced only the length of the germ tube to a certain extent, but those, like dl-threonine, dl-serine, glycine, and dl-alanine, were totally inhibitory to the germination of the spores. The hormones did not affect germination except for gibberellic acid and indol-butyric acid which were slightly stimulatory to the growth of the germ tube. Complete inhibition was observed in 2, 4-D. In general, the response of the light type isolates to the external supply of nutrients was more than that of the dark type ones.

Acknowledgements : Gratefulness is acknowledged to Drs. B. L. Chona and D. N. Srivastava for the helpful advice given to me during the course of these investigations, and to Dr. V. V. Chenulu for critically going through the manuscript.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi-12.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

CHATRATH, M. S. AND B. S. BAJAJ (1964) studies on the physiology and pathogenicity of an avirulent strain of *Colletotrchum falcatum* Went. *Indian J. Sugarcane Res. & Dev.* **8** : 288-292.

COCHRANE, V. W. (1958) *Physiology of Fungi*. John Wiley and Sons, Inc., New York, 524. pp.

COOPER, W. C. (1939) Vitamins and germination of pollen grains and fungus spores. *Bot. Gaz.* **100** : 844-852.

DAVIS, D. AND A. E. DIMOND (1956) Site of disease resistance induced by plant growth regulators in tomato. *Phytopathology.* **46** : 551-552.

EMERSON, M. A. (1943) Chemical activation of ascospore germination in *Neurospora crassa. J. Bact.* **55** : 327-330.

*FUCHS, W. H. AND A. GAERTNER (1958) Untersuchungen zur Keimungsphysiologie des Schwarzrostes *Puccinia graminis tritici. Arch. Mikrobiol.* **28** : 303-309.

GOTTLTEB, D. (1950) The physiology of spore germination in fungi. *Bot. Rev.* **16** : 229-257.

GREWAL, J. S. (1955) Effect of nitrogen nutrition on growth and sporulation of *Alternaria tenuis* causing core rot of apples. *Lloydia.* **18** : 74-81.

HERRICK, J. A. (1940) The carbon and nitrogen metabolism of *Stereum gausapatum* Fries *Ohio J. Sci.* **40** : 123-129.

LILLY, V. G. AND H. L. BARNETT (1951) *Physiology of fungi*. McGraw-Hill Book Company, Inc., New York 464. pp.

LIN, C. K. (1945) Nutrient requirements in the germination of the conidia of *Glomerella cingulata. Amer. J. Bot.* **32** : 296-298.

NAITO, T. AND T. TANI (1951) On the influence of phytohormones on the germination of uredospores of rust fungi. *Tech. Bull. Kagawaken Agric. Coll.* **2** : 167-177.

*OHTSUKI, T. (1936) Untersuchungen uber die Nitritverwendung and die Nitratreduktion bei Schimmelpilzen. *Japan J. Bot.* **8** : 269-293.

PETURSON, B. (1951) Effect of growth promoting substancees on the germination of uredospores of crown rust. *Phytopathology* **41** : 1039-1040.

*Original not seen

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RAMAKRISHNAN, T. S. (1941) Studies in the genus Colletotrichum. II. Physiological studies on *Colletotrichum falcatum*. *Proc. Indian Acad. Sci.* **14** (B) : 395-411.

RYAN, F. J. (1948-49) The germination of conidia from biochemical mutants of *Neurospora*. *Amer. J. Bot.* **35** : 497-503

SINGH, PRITAM (1962) *Studies on the variations in* Colletotrichum falcatum *Went and their correlation, if any, with virulence.* Thesis approved for Ph. D. degree in Mycology and Plant Pathology at I. A. R. I., New Delhi, pp. 273.

SINGH, PRITAM (1964) Studies on the enzyme sucrase as secreted by the conidia of *Colletotrichum falcatum* Went. *Phytopath. Z.* (in press).

SINGH, PRITAM (1965) Amylase and polyphenol oxidase production by the germinating conidia of *Colletotrichum falcatum* Went. *Indian Phytopath.* **1** : (in press).

SINGH, PRITAM (1965) Studies on the stimulation of conidial germination in *Colletotrichum falcatum* Went. *Indian Phytopath.* **18** : 291-302.

TANDON, R. N. AND J. S. GREWAL (1956) Effect of nitrogen nutrition on growth and sporulation of some pathogenic fungi *Proc. Indian Acad. Sci.* **44** : 61-67.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE EFFECT OF CALCIUM, MAGNFSIUM, AND SULPHUR NUTRITION OF GUAR PLANTS ON LOCAL LESION FORMATION BY SUNNHEMP MOSAIC VIRUS

K. S. M. SASTRY*

(Accepted for publication December 21, 1965)

INTRODUCTION : Effect of nitrogen, phosphorus and potassium nutrition of guar (*Cyamopsis tetragonoloba* (L.) Taub.), plants on local lesion formation by sunnhemp mosaic virus (SMV) has been studied by Sastry 1962. Sastry (1963) also reported the influence of darkness on guar plants and its effect on local lesion formation. In this communication, the results of calcium, magnesium and sulphur nutrition of guar plants on local lesion formation by the Delhi isolate of sunnhemp mosaic virus (Raychaudhuri, *et al.*, 1962) resembling in its properties the southern sunnhemp mosaic virus (Capoor 1962) are reported.

MATERIALS AND METHODS : The method followed for raising the guar seedlings and the nutrient solution used was the same as described earlier (Sastry 1962), except for the different levels of calcium chloride, magnesium sulphate and sodium sulphate. The procedure followed for the preparation of the inoculum and inoculation of guar leaves was the same as described by Sastry and Vasudeva (1963). A batch of fifty guar leaves was inoculated with the clarified standard extract obtained from mosaic infected sunnhemp plants and the height, fresh weight and the number of local lesions produced were recorded.

EXPERIMENTAL RESULTS : *Calcium* : To study the effect of calcium nutrition, seedlings of guar raised in quartz sand were supplied with 100 ml. of nutrient solution containing 0,20,200,500 and 1000 p. p. m. calcium twice a week. Fifty leaves of guar approximately of the same size were inoculated under each treatment when the plants responded to the different levels of calcium supplied. Height, fresh weight and the number of local lesions recorded are set out in Table I. The results clearly indicate that the growth of the guar plants progressively increased with increased calcium supply up to 200 p.p.m. and at higher levels growth was retarded. The plants supplied with 0 and 20 p.p.m. calcium exhibited stunted growth, deformation of the young leaves, finally resulting in death of actively growing buds, typical of calcium deficiency. The number of local lesions produced remained more or less the same at all the calcium levels except at deficient levels (0 and 20 p.p.m.) where there were less number of lesions.

Magnesium : Guar plants were supplied with nutrient solution containing 0,0.5,5,48 and 486 p.p.m. magnesium and fifty leaves of

*Present Address : Scientist, Plant Pathology, Regional Research Laboratory Jammu-Tawi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

almost similar size were inoculated with sunnhemp mosaic virus. Height, fresh weight and number of lesions recorded are presented in Table II. From the results it is evident that the best growth of sunnhemp plants was at 5 p.p.m. magnesium level, beyond which (48 and 486 p.p.m.) the plants exhibited stunted growth. Plants grown at 0 and 0.5 p.p.m. levels were also stunted in growth in addition to wedge shaped green area at the base of the older leaves indicating magnesium deficiency. Maximum number of local lesions due to SMV were also recorded in plants which received 5 p.p.m. magnesium. Both above and below this level there was a decrease in the number of local lesions.

Sulphur : Five levels of sulphur *i.e.* 0,2,32,256 and 1024 p.p.m. were supplied to guar plants in the form of nutrient solution. The plants thus raised were inoculated when their response to different levels of sulphur was evident. Experiments were replicated thrice and the results are given in Table 3.

The plants grown at 0 and 2 p.p.m. were pale yellow in colour exhibited poor growth, slight curling and yellowing of the apices of the young leaves. Stunted growth was also observed in plants supplied with 256 and 1024 p.p.m. sulphur and in addition the leaves were dark green in colour. Maximum growth was recorded in plants grown at 32 p.p.m. Similarly the number of local lesions produced due to SMV increased with the increased sulphur nutrition up to 32 p.p.m. beyond which the growth of the plants was retarded and the number of lesions produced at deficient (0 and 2 p.p.m.) and excessive levels (256 and 1024 p.p.m.) was less. Further the lesions produced in plants at deficient sulphur levels were comparatively bigger in size than those produced in plants receiving optimum and excessive doses of sulphur.

DISCUSSION : Present studies have indicated that the growth of the guar plants increased progressively with the increase in calcium supply upto 200 p.p.m. beyond which the growth of the plants was retarded. The number of local lesions due to SMV produced at 200,500 and 1000 p.p.m. remained more or less same without any appreciable difference, when compared to very few number of lesions produced in plants receiving deficient levels of calcium. Similar results with regard to calcium nutrition have been reported by chessin and Scott (1955) and Penzer (1957) on *N. glutinosa* and *Phaseolus vulgaris* respectively by TMV.

With magnesinm nutrition guar plants have shown progressive increase in growth upto 5 p.p.m. and at 48 and 486 p.p.m. levels the plants exhibited stunted growth. However, there was no appreciable difference in number of lesions produced at deficient (0 and 0.5 p.p.m.) and optimum levels (5 p.p.m) But number of lesions produced in plants at excessive doses of magnesium (48 and 486 p.p.m.) were consistantly very few. Similar results have been reported by Chessin and Scott (1955) and Penzer (1957).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Effect of calcium nutrition of guar plants on the production of local lesions by sunnhemp mosaic virus.

Calcium supplied in ppm.	Experiment I					Experiment II			Experiment III
	*Height in cm.	*Fresh Wt. in gm.	No. of lesions produced **per leaf	*Height in cm.	*Fresh wt. in gm.	No. of local lesions produced **per leaf	*Height in cm.	*Fresh wt. in gm.	No. of local lesions produced **per leaf
0	16.00	2.00	9	15.25	1.75	13	14.50	1.50	11
20	17.50	2.95	13	16.90	2.15	14	15.60	2.00	15
200	20.05	4.20	40	21.15	4.50	38	22.15	5.00	43
500	19.00	3.15	32	17.85	3.00	33	18.05	2.85	38
1000	15.50	1.95	35	16.00	2.00	30	15.10	1.80	33

*Average for 15 plants.
**Average for 50 leaves.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2 : Effect of magnesium nutrition of guar plants on the production of local lesions by sunnhemp mosaic virus.

Magnesium supplied in ppm.	Experiment I			Experiment II			Experiment III		
	Height in cm.	*Fresh weight in gm.	**No. of local lesions produced per leaf	*Height in cm.	*Fresh weight in gm.	**No. of local lesions produced per leaf.	*Height in cm.	*Fresh weight in gm.	**No. of local lesions produced per leaf.
0	14.15	1.50	35	13.55	1.45	32	16.10	2.00	40
0.5	16.30	2.00	33	16.75	2.35	39	18.25	3.15	43
5	20.35	4.85	40	22.10	5.15	45	23.85	5.25	48
48	15.25	2.15	20	18.15	2.20	26	17.00	2.85	25
486	12.05	1.05	16	11.15	1.55	18	15.15	1.95	19

*Average for 15 plants.
**Average for 50 leaves.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3 : Effect of sulphur nutrition of guar plants on the production of local lesions by sunnhemp mosaic virus.

Sulphur supplied in ppm.	Experiment I				Experiment II		Experiment III		
	*Height in cm.	*Fresh wt. in gm.	*No. of local lesions produced per leaf.	*Height in cm.	Fresh wt. in gm.*	**No. of local lesions produced per leaf.	*Height in cm.	*Fresh wt. in gm.	**No. of local lesions produced per leaf.
0	15.20	2.15	16	16.15	2.60	18	14.75	1.50	15
2	17.00	3.25	25	18.50	3.90	18	16.15	3.15	27
32	22.15	5.65	36	23.00	5.75	46	22.55	4.95	39
256	18.05	4.00	20	17.55	3.55	19	16.85	3.25	20
1024	16.15	3.25	12	15.90	3.00	15	15.75	2.85	10

*Average for 15 plants.

**Average for 50 leaves.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The supply of sulphur has shown that the maximum growth of the plants can be obtained at 32 p.p.m. and at deficient and excessive levels the growth of the plants was retarded. Similarly the maximum number of local lesions due to SMV were also produced in plants supplied with 32 p.p.m. Thus it clearly indicates a positive correlation between the growth of the plants and the number of lesions produced. These findings are similar to those of Chessin and Scott (1955) and Penzer (1957).

Summary

Growth of guar plants progressively increased with increased calcium supply upto 200 p.p.m. and at higher levels there was retardation of growth. The number of local lesions produced by SMV remained more or less the same at all levels of calcium except at deficient levels (0 and 20 ppm). Maximum growth as well as local lesions due to SMV in guar occured at 5 p.p.m. of magnesium nutrition. Similarly in case of sulphur nutrition 32 p.p.m. supported maximum growth as well as highest number of local lesions due to SMV.

Acknowledgement : The author is very much indebted to Dr. B.L. Chona, Head of the Division of Mycology and Plant Pathology for the facilities provided during these studies. The author is also indebted to the Council of Scientific and Industrial Research (Scientists' Pool) for the financial help.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute
New Delhi—12.

References

Capoor, S.P., (1962) Southern Sunnhemp mosaic virus a strain of tobacco mosaic virus. *Phytopathology* **52** : 393-97.

Chessin, M. and H.A. Scott, (1955) Mineral nutrition and the size of local lesions induced by tobacco mosaic virus. *Science* **212** : 3134, 112.

Chessin, M. and H.A. Scott, (1955) Calcium deficiency and infection of *Nicotiana glutinosa* by tobacco mosaic virus. *Phytopathology* **45** : 288-290.

Penzer, J.S. (1957) The effect of mineral salts on local lesions formation by *Phaseolus vulgaris* inoculated with tobacco mosaic virus. *Phytopathology*, **17** : 448-751.

Raychaudhuri, S.P., T.K. Nariani and C.R. Dass (1962) *Cyamopsis tetragonoloba* (L.) Taub. a local lesion host of sunnhemp mosaic virus. *Indian Phytopath.* **15** : 79-80.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SASTRY, K.S.M., (1962) Effect of mineral nutrition on local lesion formation by sunnhemp mosaic virus in guar. *Indian Phytopath.* **15** : 254-458.

SASTRY, K.S.M. (1963) Effect of darkening on local lesion formation in guar *Cyamopsis tetragonoloba* (L.) Taub. by sunnhemp mosaic virus. *Indian J. Micro. Biol.* **3** : 67-72.

SASTRY, K.S.M. (1964) Effect of trace elements on multiplication and concentration of sunnhemp mosaic virus. *Indian J. Expt. Biol.* **2** : 185-189.

SASTRY, K.S.M. AND R.S. VASUDEVA (1963) Effect of host plant nutrition on multiplication and concentration of sunnhemp mosaic virus. *Indian J. Agric. Sci.* **33** : 118-128.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

OCCURRENCE OF PHYSIOLOGIC RACES OF WHEAT AND BARLEY RUSTS IN INDIA DURING 1957-62

R. PRASADA, V. C. LELE, L. M. JOSHI, SHEODHAN SINGH, M. M. PAYAK, D. P. MISRA, G. SWARUP, L. B. GOEL, G. KRISHNA AND S. K. SHARMA

(Accepted for publication January 9, 1966)

Detailed accounts of the occurrence, prevalence and distribution of physiologic races of wheat and barley rusts in India have been published from time to time in this country (Mehta, 1933, 1940 ; Vasudeva *et al.*, 1955, 1960 and 1961). The last comprehensive account of the physiologic races dealing with the period 1952-57 was published in 1961 by Vasudeva *et al.* In the present account information up to the end of 1962 is being presented. The method of obtaining samples and their study is same as discussed earlier and hence it is unnecessary to repeat them here.

During 1957-62 there has been a gradual rise in the number of samples of black or stem rust [*Puccinia graminis* var. *tritici* (Pers.) Eriks. and Henn.] and brown rust (*P. recondita* Rob. ex Desm.) and on an average 250 samples of each rust were analysed as against 150-200 samples in earlier years. However, in the case of yellow rust (*P. striiformis* West.) of wheat the number has not increased correspondingly. Taking all the 3 rusts of wheat and barley together, a total of 2409 collections was analysed during these five years and their account is furnished here.

A close study of data collected during last 30 years or so has confirmed the earlier views that there is no regional distribution of races of black and brown rusts in this country, and this may be due to the absence of natural barriers such as high mountains in the sub-continent. In the Indo-Gangetic Plains, which is the chief wheat-producing region of the country, all the 3 rusts are prevalent. Stripe or yellow rust (*Puccinia striiformis*) is more destructive in the areas adjacent to foot-hills of Himalayas and it gradually diminishes in its prevalence away from the foot-hills towards the south, so much so that it is practically absent from Peninsular India except for Nilgiri and Pulney Hills. Black and brown rusts are prevalent in all parts of the country wherever wheat is cultivated. Recently, brown rust has become very frequent and is appearing in a destructive form even in those parts of the country year after year where it was insignificant in the past.

As already stated in the earlier publications of this series, the study of physiologic races of wheat and barley rusts was started in India as early as 1932 (Mehta, 1933) and so far (till the end of 1962) 46 races and biotypes of the 3 rusts have been isolated. The study of biotypes within races is comparatively a new field of research in this country, and was started by Uppal and Gokhale in late forties when they recorded the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

occurrence of biotypes within races 15 and 42 of black rust. All the races and biotypes so far recorded from India are listed below and the races which have been reported for the first time since 1957 have been indicated with an asterisks.

Black Rust : (*Puccinia graminis tritici*)	Races 11**, 14*, 15, 15-C, 17, 21, 21-A, 21-A-I, 24, 34, 34-A*, 40, 42, 42-B, 72, 75, 117, 122, 194 and 117-A**.
Brown Rust : (*P. recondita*)	Races 10, 11, 16*, 17, 20, 26, 63, 70, 77, 106, 107, 108, 131*, 162, 162-A* and D*
Yellow Rust (*Puccinia striiformis*)	Races 13, 19, 20, 31, A, D, E, F, G and H.

The reactions of differential hosts to new races of Black and Brown rusts are set out in Table 1.

Distribution and frequency of occurrence of races from year to year are summarised in Tables 2-A, 2-B, 2-C, 3-A, 3-B and 3-C. The frequency of occurrence of races was estimated as in previous years on the basis of total number of times a particular race was isolated in relation to the total number of isolates studied from that crop year.

DISTRIBUTION AND PREVALENCE OF RACES :

BLACK RUSTS : From 1957-58 to 1961-62 crop seasons two new races, namely 11 and 14, and 2 new biotype of races 34 and 117 have been isolated and these are being maintained in viable condition at the Plant Pathological Sub-station, Simla (6,900 ft. a. s. l.) where the climatic conditions for their maintenance are suitable. Varieties Charter and Yalta differentiate biotypes of races 34 and 117 from the type races by being susceptible to the biotypes (Singh, 1962 ; Patil *el al.*, 1963). Apart from these new races, a number of races reported earlier were also isolated. On the other hand, certain races like races 72, 75 and 194 were not met with at all. Race 75 has not been met with since 1937 (Vasudeva *et al.*, 1955, 1960 and 1961) and has obviously become extinct. It was one of the four races originally picked up in two collections of 1930-31 in combination with races 40 and 42 (Mehta, 1940). This race showed certain morphological peculiarities and had an incubation period about one and half times more than that of other races (Joshi and Kak, 1958). Similarly, race 194, first isolated in 1945 by Prasada and Lele (1952) has not been met with even once during these past fifteen years (*i. e.* since 1947-48 crop year). In fact, it was met with only between 1944-47 and that too very rarely. Both these races were weak in their pathogenic range and probably this is the reason for their absence.

A disquieting feature revealed by these studies is the gradual increase in the prevalence of race 122. This race is the most destructive race in India and we have, at present, not a single commercial variety which can effectively resist the race if it increases in its prevalence.

*New races, isolated for the first time in this country from the samples collected between 1957-62.

**Races reported by Patil *et al.*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Prasada and Joshi (1961) have pointed out the danger we are confronted with and they have stressed upon the possibility of its becoming a widely prevalent race.

Among the remaining races of the rust, only four races and their biotypes (namely, 15, 15-C, 21, 21-A, 21-A-l, 40, 42 and 42-B) were the most prevalent ones and are discussed below. For the sake of convenience the races and their biotypes have been dealt with together.

Race 15 : This is one of the few races in this country that has shown significant fluctuations in frequency since inception of the physiological race studies in 1930 (Vasudeva *et al.*, 1952). It was one of the first four races to be isolated in India from 1931-32 collections (Mehta, 1933) and occupied the first rank in prevalence in the thirties as well as in early forties (upto 1943-44). Then followed a period of gradual decline and it fell so much that it was not met with at all from 1949-50 to 1952-53. Since 1953 there is again a gradual rising tendency and this race and its biotypes are being met with frequently.

Race 21 : Like the previous race, this race too has shown considerable fluctuations in its prevalence and distribution. However, the fluctuation curves of both of them are opposite. In earlier years between 1930 to 1943 when race 15 was most widely prevalent, this race was almost rare. But later there was a sudden increase in its prevalence so much so that for eight years (1947-48 to 1955-56) it remained the predominant race and side by side there was a corresponding decline in the frequency of race 15. Race 21 and biotypes still form the bulk of isolates and are widely prevalent throughout the country.

Race 40 : Like the above mentioned races, race 40 also has shown considerable fluctuations from time to time. Mehta (1940) isolated this race as early as 1931 and therefore, it is one of the earliest recorded races in this country. Since then this race has been met with every year. The race attained the first position in prevalence from 1938 to 1941 and again from 1957 to 1960.

Race 42 : Race 42 and its biotypes have always been considered important because they infect the *dicoccum* varieties of wheat which are cultivated in Peninsular India. In recent years 3 more biotypes and sub-biotypes of this race have been reported. In 1957-62 race 42 and its biotypes were comparatively less prevalent than races 21 and 40, nevertheless they remain most important races to be reckoned with.

BROWN RUST : In brown rust, race position has shown significant changes during the last ten years or so. Some races like races 77 and 162, which were isolated after 1955 have come to occupy the foremost position while other races like race 63 which were very well established in the past have either dwindled down to insignificant position or much lower prevalence to-day.

Race 20 : It became a very important race during 1953 to 1957 and, in certain years, comprised about half the number of the isolations. However, since 1957 there was a decline and the race, though widely distributed, is not so much prevalent as it was before.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Race 63 : Like the preceding race, 63 has shown a gradual decline and in certain years its frequency has even been negligible. It was an important race up to 1950 but since then it has shown a gradual downward trend.

Race 77 : Race 77 was first isolated in 1954 (Vasudeva *et al.*, 1955) and since then it has shown increasing prevalence. This race is particularly significant as it can infect all the differentials of leaf rust including Mediterranean and Democrat. From the point of view of prevalence it may to-day be regarded as one of the first four races of *Puccinia recondita*.

Race 162 : Isolated from 1956-57 crop, this race has almost leaped to the first position by 1961-62. Very few races have shown such sudden increase from 0.55 per cent to 38.7 per cent within a period of five years. During the period under report it has remained the most widely distributed race. "Thew" is resistant to race 162 but susceptibe to its biotype.

YELLOW RUST : It is rather difficult to draw a clear picture of the races prevalent in case of *Puccinia striiformis* for the simple reason that comparatively only few samples were analysed. The only conclusion that can be drawn from the available data is that, as in the past, races A, 19 and 31 have remained the most widely distributed races and were isolated frequently.

DISCUSSION : The possibilities of appearance of new races in rusts have been discussed in detail in the earlier papers (Vasudeva *et al.*, 1961 ; Vasudeva and Joshi, 1958) and need not be repeated here. Since the publication of these articles no further information, which may elaborate these points has been collected. Preliminary work carried out on heterocaryosis has so far given negative results. Search for the occurrence of natural infection of wheat rusts on *Berberis* in Nepal and Simla Hills has not added anything more to our knowledge except to the belief that at certain suitable places high up in the Himalayas infection of *Berberis* is possible. This possibility has been emphasised by many workers from time to time (Vasudeva, 1953 ; Vasudeva and Joshi, 1958; Vasudeva *et al.*, 1960). It is worthwhile to point out here that even Dr. K. C. Mehta never ruled out this possibility as is evident from the following quotation : "Judging from the small number of races found so far it appears that as in Australia, the formation of races as a result of infection on barberries in this country occurs very rarely, if at all" (Mehta, 1940, p. 162).

More extensive search may some day lead to the discovery of aecidia of black rust of wheat on *Berberis*. If found, this will furnish scientific evidence for the appearance of new races which so far has been in the realm of theoretical speculation. However, such a discovery may not materially change our present concepts of annual recurrence of the rust in the plains of India and its control.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : The reactions of differential hosts to new races of Black rust

Race	1st isolated in	Little Club	Marquis	Reliance	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli	Reference
11	1962	3	4	3-4	2-3	3-4	4	3	3-4	3-4	3	0-1	1	Patil, (1962)
14	1959	4	0 ;-2	0 ;	0 ;	4	4	4	4	4	3-4	0 ;-1	0 ;-1	Singh (1960)

The reactions of differential hosts to new races of Brown rust

Races	1st isolated in	Malakof	Carina	Brevit	Webster	Loros	Mediterra-nean	Hussar	Democrat	Reference
16	1961	0 ;	1-2	1-2	0 ;-1	2-3	0 ;-1	0 ;	0 ;	Payak (1965)
131	1959	0 ;	4	4	0 ;-1	4	0 ;	0 ;	0 ;	Payak (1965)
D	1962	0 ;	4	0 ;-1	0 ;-3	0 ;	0 ;	0 ;	0 ;	Misra and Sharma(1963)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2-A : Occurrence of physiologic races of *Puccinia graminis* var. *tritici* in different states during 1957-1962

Crop years : Name of the States	1957-58		1958-59		1959-60		1960-61		1961-62	
	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with
Himachal Pradesh	8	17,21,21-A, 40,42B	8	21,17,34	17	21A,34,40, 117,15C	45	40,17,21A, 21-A-1,42, 42-B,21,15, 34A,34,117, 15C	22	21-A-1, 40,21, 21-A,34A, 34,42,122
					2*	..... 21,21A	3*	..... 21A,40,42, 42B,24		
Punjab	15	15,17,21,40, 42-B,21-A, 21-A-1,34	14	21,24,17,14, 21-A-1,34, 40,42	12	15,21-A,21, 34,40	43	21-A,40,17, 21,24,34, 21-A-1,42-B, 117,15	40	40,21-A, 21-A-1, 34-A,42-B, 15-C,21, 122
			1*	 40	1*	 21			1*	 21,24
Delhi	6*	21,34,40,42-B	2	17,21,21A, 34, 42B	...	...	5	15,21A,117	4	21,24, 21-A-1, 21A
							1*	..... 21A		
Jammu and Kashmir	6	17,21,21-A-1, 40	1	40	...	...	...	...	...	...

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Uttar Pradesh	59	15,17,21,21-A, 12,21-A-1,34, 24,40,42-B,15C	32	21,21A, 21-A-1,24,15, 34,40,17,117	25	21,40,15C, 21A,24,34, 42-B	26	21-A,34,40, 42B,117,24, 122,14,21-A-1	23	21,21A, 21-A-1,40, 42,17,24
	4*	21-A,40,42-B	1*	14,24	2*	15C,21A	5*	17,21,40,21A, 117	3*	17,21 21-A-1
Bihar	9	15,21,21-A, 21-A-1,34,40, 42-B	13	17,34,40,21A, 15,24,15-C	3	34,40	16	15C,34,34A, 42B,21-A, 21-A-1,40,24, 117	9	40,42,122 21A,34A, 42B, 21A-1,117
	1*	21,40,42-B					4*	21-A,15C,117		
Orissa	4	15,15C,21, 21-A,34,42	2	14,21,40	4	14,15C, 21-A	...	...	5	21-A, 21-A-1,21 40
West Bengal	6	17,21,40,42-B	2	24,34	1	40	3	21-A,21-A-1, 42-B,117	3	21-A, 21-A-1
			1*	40						
Assam	...	...	..	...	1	40	...	...	...	...
Madhya Pradesh	6	15,21,40,24, 21-A,42-B	25	40,14,24, 21-A-1,21A, 34,15,17	17	40,17,42-B, 24-34	6	24,17,42,21-A, 42-B,34,117	52	40,34A, 21A-1, 15C,24,42, 117,15,17, 42B
			2*	17,24	1*	40			3*	21-A-1, 24,40

Contd.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Contd.

Rajasthan	...	...	1	14	1	40	11	17,40,117, 24,42	...	...
							1*	117		
Bombay (Maharashtra)	40	15,17,21,24, 34,40,21-A, 42,42-B,122, 15C,21-A-1	115	24,40,42, 42-B,15,21, 14,34,17, 21-A-1,15C	88	15C,17,24,40, 15,34,42-B, 21A,14,42,122	7	40,42,117	19	15,15C, 40,42,17, 24,117, 122, 21-A-1, 14,21
	1*	17,40	1*	24,40						
Andhra Pradesh	2	40,42-B	3	14,40	2	42-B	21	42-B,21-A, 40,117	...	...
Mysore	7	15C,24,40, 42-B	19	14,24,34,40, 42B,17,15,21, 21-A-1,117	27	40,42,15,24, 21A,15C,17, 42B,14,117, 122,34	15	42,42B,117, 42B,17,15C	7	17,24,24, 42,15C, 42B,117
Madras	8	15,21-A-1, 24,40	10	21-A-1,42B, 21,34,40,15	2	21A,24	16	40,15,34A,117, 42B,21-A-1, 42,17	3	40,42 21-A-1
	1*	40								
Gujarat	...	...	...	...	...	...	1	21-A	11	21-A-1, 34-A,40
Kerala	...	...	...	...	...	...	...	...	...	...
	...	...	...	...	...	...	...	...	...	...
Nepal	...	...	...	...	...	...	9	15C,21-A, 21-A-1,21,24, 40,42-B,42	...	...
							1*	40		
Afghanistan	...	...	1	40	...	...	...	...	...	...
Total	184		254		208		230		206	

*Barley samples

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2-B : Distribution of physiologic races of *Puccinia recondita* in different states during 1957 to 1962

Crop years Name of the States	1957-58		1958-59		1959-60		1960-61		1961-62	
	No. of samples	Races met with	No. of samples	Race met with	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with
Himachal Pradesh	9	10,20,26, 162,77	8	10,20,162,63	7	63,162,106	29	10,11,20,162, 77,106,63	25	10,20,63,26 77,162,162 biotype,107
Punjab	11	10,11,77,162, 20,106,107, 17	14	20,63,162,77, 107,10,106	34	10,20,77,162, 63,107,131	13	10,20,77,162	43	10,20,63,77, 26,162, 162 biotype, D,106,70
Delhi	7	20,77,162	10	10,77,162,20	10	10,20,77,107	5	10,20,77	4	162
Jammu and Kashmir	6	10,20,162	...	...	1	77	1	162	...	...
Uttar Pradesh	60	10,20,17,162, 77,70,63,108, 11,107,106	81	10,11,77,63, 106,17,162, 20,107,108	51	10,77,162,20, 162 biotype, 108,11,106,63	61	10,20,77,162, 63,107	68	20,63,77, 162, 162 biotype,107, 106,108
Bihar	8	20,77,108,11, 162,20	26	63,10,77,20, 107,162,106, 131	14	10,17,77,20, 162,106	20	162,20,106,10, 107,77	23	10,20,63, 77,106,162, 162 biotype
Orissa	7	20,26,77,162	3	26,162	5	20,26,77	...	...	21	63,77,162, 20,108,10

Contd.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Contd.

West Bengal	21	20,77,162,11, 10,26,63,106	14	77,162,10,17, 63,106	7	20,162	13	10,20,162,77	5	162
Madhya Pradesh	1	20	6	10,20,106	13	70,20,107,10	92	10,162,77,107, 26,162 biotype 106,108,20	18	20,63,106, 77,162
Rajasthan	1	11	5	10,20,77,162	2	10	...	...	2	20,107
Bombay (Maharashtra)	22	63,107,26,77, 20,63,162 10,11	9	10,20,131,107, 63,162	55	10,20,77,107, 17,106,131	9	10,63,77,107	15	20,77,162
Andhra Pradesh	...	...	...	...	...	...	1	20,77	...	...
Mysore	21	107,20,26,10, 162,17,63, 162,10,11	9	10,20,131,107, 63,162	55	10,20,77,107, 17,106,131	9	10,63,77,107	15	20,77,162
Madras	8	26,107,162, 11,20,10,77	7	162,20,106, 26,107,63	8	16,77,107, 162,106	7	10,63,77,106,	3	20,26,162
Gujarat	...	...	...	...	12	20,107	...	...	27	20,77,106, 26,162,63
Kerala	...	...	1	11	...	...	...	...	...	...
Nepal	...	...	...	...	...	...	15	11,63,106, 162,77,107	...	...
Afghanistan	...	...	1	10	...	...	...	...	...	...
Total	182		189		229		191		255	

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2-C : Distribution of physiologic races of *Puccinia striiformis* in different states during 1957-1962

Crop years / Name of the State	1957-58		1958-59		1959-60		1960-61		1961-62	
	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with	No. of samples	Races met with
Himachal Pradesh	24	13,19,31,A, 20,H		13,31,20,A	16	13,20,A,31	...	...	...	...
		.....		.....		.....				
	4*	19	10*	A,G,31	9*	A,G,19,31				
Punjab	31	A,31,19,20	20	13,19,31, 20,A	24	13,20,31,A	10	19,20,31,A,	6	A,E,31
		.....		.....				.....		.....
	4*	19,G	4*	19,31,G			1*	19	1*	19,31
Delhi	4	13,19	1	20	...	...	...	...	...	...
				.....						
			1*	G						
Uttar Pradesh	9	19	7	13,31,19, A,D	3	13,20	11	19,A,31	...	...
		.....		.....		.....		.....		
	2*	G,19	2*	G,19	8*	19	16*	G,19		
Bihar	2	19	1	19	1*	19	1	19	...	...
								.....		
							1*	19		
Orissa	1*	G	...	...	...	...	...	...		
West Bengal	2	13,19	...	...	...	...	...	...	...	...
Madhya Pradesh	1	19	...	...	...	...	1	19	...	...
								.....		
							1*	31		
Madras	2	A,G	1	13	3*	G	...	...	...	...
		.....		.....						
	11*	G	1*	G						
Total	97		71		64		42		7	

*Barley samples

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Frequency of races in percentage

TABLE 3-A : *Puccinia graminis* var. *tritici*

Races	11	14	15	15-C	17	21	21-A	21-A-1	24	34	34-A	40	42	42-B	117	122	75	194
Year																		
1957-58	—	—	7.1	1.9	7.1	17.3	5.5	6.7	4.3	6.7	—	31.5	0.7	9.8	—	0.39	—	—
1958-59	—	10.0	6.7	0.58	3.5	8.2	2.05	3.2	16.4	10.8	—	28.5	1.16	6.4	1.16	0.29	—	—
1959-60	—	2.1	4.3	7.01	3.5	3.07	9.21	—	8.6	6,57	—	45.9	5.2	5.7	2.86	0.87	—	—
1960-61	—	0.35	2.11	1.4	3.5	5.2	23.3	3.5	2.4	4.6	2.11	14.0	15.2	8.4	27.1	0.35	—	—
1961-62	0.42	0.42	2.5	2.9	2.9	6.7	4.2	29.4	5.04	0.84	4.6	24.7	5.9	2.1	3.3	2.1	—	—

TABLE 3-B : *Puccinia recondita*

	10	11	16	17	20	26	63	70	77	106	107	108	131	162	162-A	D
1957-58	12.05	6.2	—	1.7	16.9	5.3	8.03	0.44	15.6	1.7	9.3	0.88	—	21.4	—	—
1958-59	24.0	3.1	—	1.32	9.5	0.88	7.9	—	12.0	4.4	3.5	0.44	0.88	31.4	—	—
1959-60	14.5	0.39	0.79	1.9	15.6	1.5	2.7	—	11.5	2.7	26.2	0.39	0.78	19.6	1.17	—
1960-61	14.9	1.7	—	—	10.4	0.43	3.07	—	15.3	3.9	6.5	0.43	—	42.1	0.86	—
1961-62	2.2	—	—	—	25.3	1.12	11.21	0.37	12.7	2.6	1.4	0.72	—	38.7	4.1	0.37

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 3-C. *Puccinia striiformis*

Races	13	19	20	31	A	D	E	F	G	H
Year										
1957-58	5.05	40.4	4.04	13.1	20.2	—	—	—	16.16	1.01
1958-59	11.2	15.4	16.8	23.9	21.1	1.4	—	—	9.8	—
1959-60	3.1	15.6	23.5	23.5	18.7	—	—	—	10.9	—
1960-61	—	45.2	4.76	23.8	19.04	—	—	—	7.14	—
1961-62	—	12.5	—	25.0	37.5	—	25.0	—	—	—

REFERENCES

GOKHALE, V. P. (1950) A new biotype of race 15 of *Puccinia graminis*. *Curr. Sci.* **19** : 214-215.

JOSHI, L. M. AND D. KAK (1958) Certain morphological peculiarities in races 34 and 75 of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. *Indian Phytopath.* **11** : 194-196.

MEHTA, K. C. (1933) Rusts of Wheat and Barley in India. *Indian J. agric. Sci.* **3** : 939-962.

MEHTA, K. C. (1940) Further studies on cereal rusts in India. *Sci. Monogr.* No. 14. *Indian Coun. Agric. Res. India* 1-240.

MISRA, D. P. AND S. K. SHARMA (1963) A new race of *Puccinia recondita* Rob. ex. Desm. in India. *Indian Phytopath.* **16** : 313.

PATIL, B. P. AND V. P. BHIDE (1962) Occurrence of race 11 of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. in Maharastra. *Indian Phytopath.* **15** : 220-221.

PATIL, B. P., M. L. MULKEKAR AND M. SULAIMAN (1963) On the occurrence of biotype 117-A of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. in India. *Indian Phytopath.* **16** : 244-245.

PAYAK, M. M. (1965) On two unrecorded Races and a New Biotype of *Puccinia recondita*. *Indian Phytopath.* **18** : 25-27.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PRASADA, R. AND L. M. JOSHI (1961) Race 122 of Black Rust—A source of Potential danger in India. *Indian Phytopath.* **14** : 23-25.

PRASADA, R. AND V. C. LELE (1952) New Physiologic Races of Wheat Rusts in India. *Indian Phytopath.* **5** : 128-129.

SINGH, S. (1962) A virulent biotype of race 34 of *Puccinia graminis* var. *tritici* Eriks. and Henn. in India. *Indian Phytopath.* **15** : 162-163.

SINGH, S., V. C. LELE AND R. S. VASUDEVA (1960) A new physiologic race of *Puccinia graminis tritici* (Pers.) Eriks and Henn. in India. *Indian Phytopath.* **13** : 182-183.

UPPAL, B. N. AND V. P. GOKHALE (1947) A new race of *Puccinia graminis tritici* and two biotypes of race 42. *Curr. Sci.* **16** : 61.

VASUDEVA, R. S. (1954) Presidential Address. Seventh Annual General Meeting of Indian Phytopathological Society. *Indian Phytopath.* **7** : 82-87.

VASUDEVA, R. S. AND L. M. JOSHI (1958) A brief review of outstanding achievements in Plant Pathological Research in relation to wheat and barley rusts in India. (1950-57). *Proc. Mycological Research Workers' Conference*, I. C. A. R.-1958.

VASUDEVA, R. S., V. C. LELE AND L. M. JOSHI (1952) Occurrence of physiologic races of wheat rusts in India during 1949-50. *Indian Phytopath.* **5** : 63-65.

VASUDEVA, R. S., R. PRASADA, V. C. LELE, L. M. JOSHI AND D. KAK (1955) Prevalence of Physiologic Races of Wheat and Barley Rusts in India. *Indian Phytopath.* **8** : 22-51

VASUDEVA, R. S., R. PRASADA, V. C. LELE, L. M. JOSHI AND D. P. MISRA (1960) Prevalence of Physiologic Races of Wheat and Barley Rusts in India during 1952-57. I. A. R. I., Res. Bull. No. 5.

VASUDEVA, R. S., R. PRASADA, V. C. LELE, L. M. JOSHI, D. P. MISRA AND M. H. RAO (1961) Distribution and Prevalence of Physiologic Races of Wheat and Barley rusts in India during 1952-57. *Indian Phytopath.* **14** : 61-76.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CHANGES IN THE FREE AMINO ACIDS AND C : N RATIOS OF BANANA LEAVES INFECTED WITH FUNGAL PATHOGENS

G. RANGASWAMI AND S. NATARAJAN

(Accepted for publication January 10, 1966)

The effects of fungal infections on the amino acid content of plant tissue have been studied for some diseases (Hrushovetz 1954, Chang *et al.*, 1959, Rudolph 1963, McComba and Winstead 1964). In the present study, comparisons of the free amino acid content and C : N ratios of healthy leaves and infected and healthy tissues of diseased leaves of banana were made.

MATERIALS AND METHODS : Banana plants (*Musa sapidisiaca* Jacob), variety monthan, grown in the University Experimental Farm were used for these studies. Ten month old plants with uniform growth were selected. The newly emerging central leaf prior to uncoiling, the middle leaf marked by basipetal succession along the axis, and the oldest leaf at the base were selected for the studies. Corresponding middle leaves with Sigatoka leaf spot disease incited by *Cercospora musae* Zimm., and old leaves with black spot incited by *Deightoniella torulosa* (Syd. and Ashby) Ellis, and black tip incited by *Helminthosporium gibberosporium* Curzi, were selected for comparison. In all cases such leaves which were showing symptoms of well developed spots were selected for these studies. The plants selected for these studies were free from any nematode or other root pathogens or stem infections.

Paper chromatography was employed for separating and identifying the free amino acids, following the technique described by Plaisted (1958). The healthy portions of diseased leaves were selected to correspond the same positions as the fungal spots in the diseased leaf blade. Two-dimensional chromatography on Whatman No. 1 paper was employed with butanol : glacial acetic and water (9 : 1 : 2.9 v/v/v) as the first solvent and phenol : water (8 : 2 v/v) as the second solvent system. A standard amino acid mixture, prepared according to the method of Block and Weiss (1956), was used for comparison. Dried chromatograms were sprayed with 0.5 per cent ninhydrin in acetone water 95 : 5 v/v), heated in an oven at 60°C for 10 minutes and kept in a dark chamber for 18 hr, when full colour development was obtained.

For quantitative estimation of amino acids the procedure of Demetriades (1956) was followed. The spots in chromatograms were carefuliy cut and the amino acid eluted with 8 ml. alcoholic copper sulphate solution (7.2 ml. of 75 per cent alcohol and 0.8 ml of 0.1 per cent copper sulphate). The optical density of the eluent was determined

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

with Klett Summerson colorimeter and the amino acid concentration estimated by comparing with known standards. In each case triplicate samples were collected and they were analysed and in no case large variations were obtained and hence the average of the triplicates taken for comparison.

For estimating the total carbon content of the leaves the procedure recommended by the Association of Official Agricultural Chemists (1950) was followed. The total nitrogen content of the leaf was estimated by micro-Kjeldhal method.

EXPERIMENTAL : *Free amino acid content of healthy and diseased leaves*: Healthy leaves contained 13 amino acids (Fig. 1a). Though all these 13 amino acids were found in young, middle and old leaves, there were significant variations in the concentrations of some of them. Glutamic acid and cystine concentrations decreased and alanine increased with age, while in other cases there were only narrow fluctuations (Table 1). In the diseased leaves only 4 to 8 amino acids were detected, glutamic acid, threonine, leucine(s) and lysine being absent (Fig. 1 b and c). There was a greater reduction in aspartic acid concentration, lesser reduction in alanine, and arginine, and complete absence of serine and proline in the infected tissues in respect of all the three diseases. Cystine and valine appeared only in the tissue infected by *C. musae* and not in the corresponding healthy one and likewise valine appeared in the tissue infected with *D. torulosum*, whereas in the *H. gibberosporium*-infected leaves cystine was present only in the healthy portions but not in the infected tissue (Table 1).

C : N ratios of healthy and diseased banana leaves : The C : N ratio of healthy leaf widened due to reductions in nitrogen content with age. In the diseased leaves, the healthy portion contained more carbon whereas in the infected tissue there was more nitrogen. The C : N ratio of the healthy portion of diseased leaf was narrower than in the healthy leaf and in the diseased portion it was further narrowed down.

DISCUSSION : Steward *et al.* (1960) in their studies on the physiology of banana plants as influenced by nutritional deficiencies, reported on the presence of 15 amino acids, whereas in the present studies only 13 amino acids were detected, the two undetected ones being phenylalanine and L-amino butyric acid. Perhaps this may be due to varietal variations or other cultural factors. Cystine decreased with age and as suggested by Papavizas and Davey (1963) this may have an inhibitory effect on fungal mycelium and may be responsible for resistance to infection in young leaves. The significant increase in alanine content with leaf maturity may have been by transamination activity as suggested by Webster (1959).

According to Virtanen (1961) the two primary amino acids formed by incorporating inorganic nitrogen into plant and animal tissues as well as in microorganisms are aspartic and glutamic acids. Fungal

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Free amino acid contents of banana leaves at three different stages of maturity and as influenced by infections by three different fungal pathogens.*

Amino acid.	Healthy leaf			*C. musae* affected middle leaf.		*H. gibberosporium* affected old leaf		*D. torulosum* affected old leaf.	
	Young mg.	Middle mg.	Old mg.	Health tissue mg.	Infected tissue mg.	Healthy tissue mg.	Infected tissue mg.	Healthy tissue mg.	Infected tissue mg.
Aspartic acid	26.7	22.0	29.1	12.8	6.6	19.0	12.8	11.6	0
Glutamic acid	9.9	3.1	T**	0	0	0	0	0	0
Serine	24.7	19.2	27.2	8.6	0	17.8	0	12.1	0
Glycine	11.0	15.8	10.7	0	0	0	0	9.6	5.3
Threonine	23.9	25.0	19.1	0	0	0	0	0	0
Alanine	19.7	30.1	32.1	18.0	13.6	15.7	9.4	16.7	9.5
Arginine	10.3	14.8	9.2	8.9	6.1	5.8	4.6	6.7	5.4
Tyrosine	11.3	13.7	9.7	10.8	6.3	0	0	5.1	7.1
Proline	10.9	12.3	9.9	5.8	0	5.6	0	7.0	0
Leucine(s)	6.6	5.3	4.8	0	0	0	0	0	0
Lysine	4.5	5.2	2.1	0	0	0	0	0	0
Cystine	23.1	11.2	5.9	0	5.1	9.5	0	7.6	6.2
Valine	6.9	11.2	8.7	0	7.1	6.4	4.2	0	7.7

** Data represent mg/g of leaf tissue on wet weight basis and are averages of 3 replications.
*T Trace.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

infections of banana leaf seem to upset this pathway, for glutamic acid disappeared and aspartic acid concentrations was very much reduced from infection by any one of the three fungi.

TABLE 2 : Carbon : nitrogen ratio of healthy and diseased banana leaves*.

Leaf sample	C	N	C : N
	mg	*mg*	*ratio*
Young :			
Healthy leaf	92.58	2.24	41 : 1
Middle :			
Healthy leaf	99.80	1.70	59 : 1
Healthy tissue of *C. musae* infected leaf	99.58	1.50	66 : 1
Diseased tissue of *C. musae* infected leaf	89.80	1.64	55 : 1
Old :			
Healthy leaf	100.36	1.58	64 : 1
Healthy tissue of *H. gibberosporium* affected leaf	98.20	1.44	68 : 1
Diseased tissue of *H. gibberosporium* affected leaf	82.46	1.48	56 : 1
Healthy tissue of *D. torulosum* affected leaf	83.58	1.42	59 : 1
Diseased tissue of *D. torulosum* affected leaf	89.54	1.78	50 : 1

*Data represent mg/g leaf tissue on wet weight basis, and are averages of 3 replications.

SUMMARY

Analysis by paper chromatography revealed that glutamic acid and cysteine concentrations decreased and alanine increased with maturity of banana leaf. Due to infection by *Cercospora musae*, *Deightoniella torulosa* and *Helminthosporium gibberosporium* considerable reduction, both qualitative and quantitative, in the amino acid content of the diseased leaf was noted. With maturity as well as due to fungal infections the C : N ratio of the leaf narrowed down. The diseased tissues contained more total nitrogen than the healthy tissues of diseased leaf and of healthy leaf.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Microbiology Laboratory,
Faculty of Agriculture,
Annamalai University, Annamalainagar,
Madras State.

References

Association of Official Agricultural Chemists (1950) *Official Methods of Analysis*. Ed. 7. Assoc. Official Agri. Chem. Washington, 910 p.

ASHWORTH, L. J. (1959) The relation of total sugars to susceptibility of Persian melon seedlings to *Macrophomina phaseoli*. *Abstr. Phytopathology* **49** : 533.

BIRNBAUM, D. (1962) Investigations on the nitrogen content of potato leaves and its relation to *Phytopathora* attack. *Biol. Zb.* **81** : 355-370.

BLOCK, R. J. AND K. W. WEISS (1956) *Amino Acid. Handbook*. Charles Thomas, Springfield, Illinois, 386 p.

CHANG, Y. O., C. W. MCANELLY AND J. R. VAUGHN (1959) A comparison of the amino acid content of beans produced from healthy and *Fusarium* root rot infected plants. *Plant Dis. Reptr.* **43** : 437-438.

DEMETRIADES, S. D. (1956) Chromatographic detection of free-amino acids in normal and iron-deficient plants of *Hibiscus esculentus* L. *Nature* **177** : 95.

HRUSHOVETZ, S. B. (1954) The effect of infection by *Helminthosporium sativum* on the amino acid content of wheat roots. *Can. J. Bot.* **32** : 571-575.

MCCOMBS, C. L., AND N. N. WINSTEAD (1964) Changes in sugars and amino acids and cucumber fruits infected with *Pythium aphanidermatum*. *Phytopathology* **54** : 233-234.

PAPAVIZAS, G. C. AND C. B. DAVEY (1963) Effect of sulfur containing amino compounds and related substances on *Aphanomyces* root rot of peas. *Phytopathology* **53** : 109-115.

PLAISTED, P. H. (1958) Clearing free amino acid solutions of plant extracts for paper chromatography. *Contr. Boyce Thompson Inst.* **19** : 231-244.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RUDOLPH, K. (1963) Further biochemical studies on host parasite relationship as exemplified by *Puccinia graminis tritici* I. The effect of infection on acid substance exchange. *Phytopathol. Z.* **46** : 276-290.

STEWARD, F. C., A. C. HULME, S. R. FREIBERG, M. P. HEGARTY, J. K. POLLARD, R. RABSON AND R. A. BARR (1960) Physiological investigations on the banana plant I. Biochemical constituents detected in the banana plant. *Ann. Bot. N.S.*, **24** : 83-116.

VIRTANEN, A. I. (1961) Some aspects of amino acid synthesis in plants and related subjects. *Ann. Rev. Plant Physiol.* **12** : 1-12.

WEBSTER, G. C. (1959) *Nitrogen metabolism in plants.* Row Peterson and Company, New York, 152 p.

WOLF, F. A. AND F. T. WOLF (1955) The Chemical composition of leaves of diseased tobacco : ring spot, bacterial wilt and *Fusarium* wilt. *Phytopathology* **45** : 506-508.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ON THE TAXONOMY OF THE FUNGUS CAUSING ANTHRACNOSE OF DIOSCOREA ALATA L.*

R. D. SINGH[1], N. PRASAD[2] AND R. L. MATHUR[3]

(Accepted for publication January 15, 1966)

INTRODUCTION : Morphology is one of the most important characters in delineation of species in plant pathogenic fungi. Prasad and Singh (1960) described *Colletotrichum gloeosporioides* as the causal organism of anthracnose of *Dioscorea alata*. They classified it so because of presence of globose to saucer-shaped acervuli, simple hyaline conidiophores, each bearing single oval to cylindrical, non-septate and hyaline conidium (Plate 1). In his classical attempt on species classification in genus *Colletotrichum*, von Arx (1957) proposed a key which was based on the shape and size of conidia, presence or absence of sclerotia and chlamydospores, shape and size of appressoria and acervuli on host and/or in pure cultures, and host range. However, there are reports (Small, 1926 ; Ramakrishnan 1947 ; Quinione, 1958) where the morphology of acervuli, appressoria, chlamydospores and setae etc. change when subjected to various environmental conditions. Authors' studies of anthracnose pathogen on its variability and physiology have revealed the significance of such studies on the taxonomy of the genus *Colletotrichum*. Here the results on the changes in morphology of some of the characters in nature as well as under the influence of different media, temperature, light etc. are presented.

Variability in Nature : Large number of collections of diseased material of *D. alata* was made from different localities to obtain maximum number of isolates and strains. Isolations were made both from leaf and stem pieces and single spore cultures of pathogenic isolates were maintained on 2 per cent potato dextrose agar medium.

On the basis of cultural characters, viz. amount of mycelium, spore production and acervuli formation, isolates could be separated into three groups (designated as R^1, R^2 and R^3) characterised as follows :—

R^1 Aerial mycelium scanty ; sporulation profuse, dense, continuous, cinnamon coloured masses covering most of the colony surface ; acervuli dark, scattered on the surface in later stages of growth ; setae dark brown but rarely produced.

*Part of Ph. D. thesis submitted to the University of Rajasthan, Jaipur by the senior author.

1. Assistant Plant Pathologist, Agricultural Experiment Station, University of Udaipur, Campus Jobner (Jaipur). 2. Dean, University of Udaipur, Campus Jobner (Jaipur). 3. Plant Pathologist, Department of Agriculture, Rajasthan, Jaipur.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

R^2 Aerial mycelium scanty ; sporulation profuse but not continuous, cinnamon colour in earlier stages but later grey to dark grey ; acervuli grey, big and scattered densely on the surface ; setae dark brown, produced frequently in subsequent subculturings.

R^3 Aerial mycelium moderate, dull white to olive grey ; acervuli dark, profuse, smaller than that of R^2, covered with orange cinnamon spore masses, which later on changes grey to dark grey ; setae dark brown, produced frequently in subsequent subculturings.

For a study of their virulence, inoculations were made from the three isolates on two months old plants of *D. alata*. The intensity of disease was measured by a diagram representing five categories (ratings) of disease intensity prepared on the basis of different type of spots by Singh (1964). All the three isolates were found to be highly pathogenic but there were slight differences in their incubation period and virulence.

VARIABILITY UNDER ARTIFICIAL CONDITIONS : *Variation in characters due to different media* : Eight synthetic and semisynthetic solid media were inoculated separately with single germinating conidium and the growth, sporulation, acervuli production and variation in size of conidia of isolate R^1 observed at room temperature after 8 days of growth are given in Table 1.

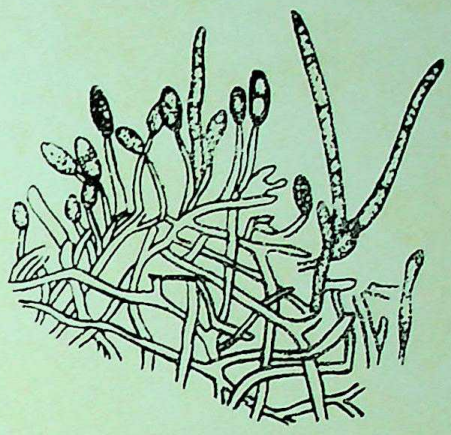
TABLE 1 : Influence of culture media on different characters of isolate R^1.

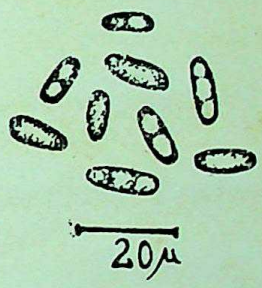
Medium	Colony dia in mm.	Sporulation	Size of spores in μ	Acervuli formation	Setae
Potato dextrose agar	56.3	+++++	15.6×4.4	Many in rings	Rare
Dioscorea dextrose agar	52.0	++++	15.9×4.4	Few and scattered	Few
Oat meal agar	40.3	+++++	18.4×3.7	Abundant in rings	Abundant
Corn meal agar	41.3	+++++	18.0×3.7	Rare	Rare
Richard's agar	31.6	++	14.5×4.1	Few and scattered	Few
Czapek's (Dox) agar	40.3	+++	17.7×3.7	Many in rings	Few
Brown's agar	30.6	+++	17.0×3.7	Many in rings	Few
Asthana Hawker's agar	30.4	+	17.7×3.7	Few and scattered	Few

Note : +++++=Excellent ; ++++=Very good ; +++=Good ; ++=Fair ; +=Poor.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri





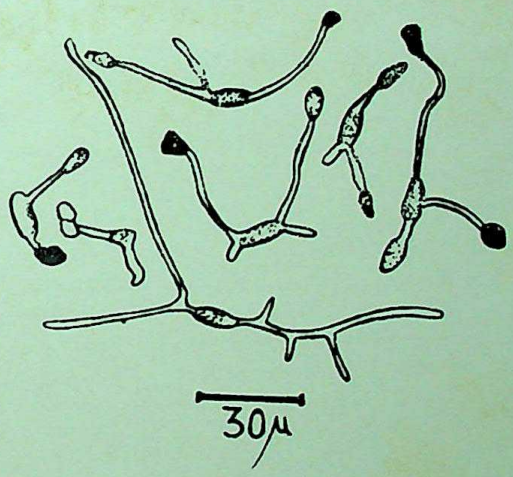


Plate 1. (A) Part of acervulus (B) Conidia (C) Germinating conidia showing the formation of appressoria.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Plate 2. Abnormal Chlamydospore formation in mycelium of *Colletotrichum* due to ammonium sulphate.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

From the above data, it is clear that growth and sporulation of the fungus was much influenced by different media. Its effect was also very conspicuous on the production of acervuli and setae and on the size of spores which on average ranged from 14.5 to 18.4μ in length.

Effect of temperature on size and production of acervuli and setae : In single spored culture plates containing 2 per cent potato dextrose agar incubated at 18°, 20°, 22°, 24°, 26°, 28°, 30°, and 32° C for 11 days the number of acervuli increased with the rise in temperature but there was a corresponding reduction in their size. Average diameter of the acervuli at 18°C. measured 237.1μ, at 28°C it was 90.8 μ, and at 30° and above their formation was not complete. Setae were produced profusely at 5°C and at higher temperatures like 30° and 32°C.

Variations in morphology as influenced by different light conditions : Effect of various sources of light on the growth, sporulation and other cultural characters of the isolate R^1 was examined. Maximum aerial mycelium was produced under total darkness while best sporulation in cultures exposed to direct sun light for three hours daily. Continuous light or darkness did not induce zonation. Largest number of acervuli were produced due to alternate light and darkness. Number of acervuli produced under total darkness was less but their average size was 272.2μ while under other treatments it did not exceed 177.0μ. Setae were not produced under conditions of total darkness but their number was very good under alternate light and darkness (diurnal light) and where cultures were exposed to direct sun light for three hours daily.

Chlamydospore formation due to ammonium sulphate : When ammonium sulphate was supplied as source of nitrogen in Czapek's solution, it was very poorly utilised by the fungus under study and caused a very abnormal development of large number of chlamydospore-like structures in the mycelium (Plate 2).

HOST RANGE OF THE FUNGUS : Seasonal plants reported to be hosts for species of *Colletotrichum* were tested for susceptibility to R^1 isolate of anthracnose fungus. They included *Mangifera indica*, *Citrus aurantifolia*, *Dolichos lablab*, *Capsicum annum*, *Spinacea oleracea*, *Cyamopsis tetragonoloba*, *Lagenaria vulgaris*, *Cucurbita maxima*, *Lycopersicon esculentum*, *Zingiber officinale*, *Sorghum vulgare* and *Zea mays*. None of the plants except *D. alata* produced any disease symptoms, exhibiting a high host specialisation of the pathogen.

In another experiment three varieties of *D. alata* and five other species of *Dioscorea* viz. *D. pentaphylla*, *D. bulbifera* var. *vera*, *D. oppositifolia* var. *linnae*, *D. esculenta* and *D. hispida* were inoculated with R^1 isolate to see their resistance to the pathogen. The results have indicated that the pathogen is very specific to its host and does not infect any species other than *D. alata* and even within the same species Magenta variety from Kerala showed some tolerance.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

DISCUSSION : Since the genus *Colletotrichum* was erected by Cordà in 1837, it had been distinguished from closely related genus *Gloeosporium* by the presence of setae in the former and their absence in the latter. Examination of large number of isolates of the pathogen causing anthracnose disease of *D. alata* revealed that mostly glabrous acervuli were produced in nature as well as in cultures but under subsequent subculturings, setae were observed to develop occasionally. Authors' study of variability has also shown that the form ation of setae is influenced by temperature, light and different substrates and as such this criterion for separating the two genera seems to be invalid.

On the basis of cultural characters three strains were screened from the isolates of *Colletotrichum* causing anthracnose of *D. alata*. However, Small (1926) found large variability and no physiologically constant feature and he did not prefer to distinguish various strains by different named species.

The formation of appressoria by the unicellular, hyaline conidia during germination was recognised to be the characteristic feature of all *Colletotrichum* species by von Arx (1957). Isolates from *D. alata* produced appressoria but mostly where the germ tubes came in contact with host surface or some other hard surface. On the other hand numerous chlamydospore type of structures developed when ammonium sulphate was supplied as source of nitrogen to anthracnose fungus. Such observations were also made by Biraghi (1934) who drew an analogy between appressoria and chlamydospores and has treated appressoria as organs of conservation, produced under unfavourable environmental conditions. Quinione (1958) studied appressoria in 27 species of *Colletotrichum* and found all sorts of variations in their size and shape. Hence shape and size and production of chlamydospores and appressoria cannot be accounted for species classification in *Colletotrichum*.

Present studies have shown that the size of acervuli which is one of the other bases of species classification in the key proposed by von Arx (1957) fluctuates very much under the influence of different media, temperature and light. These observations are in agreement with those made by Ramakrishnan (1947) and Ling and Lin (1944). Thus size of acervuli and its presence or absence can not be taken basis for species differentiation in this genus.

A great emphasis has been laid by von Arx (1957) on the size of conidia in maintaining a number of species. As indicated in Table 1, size also varies under different nutrient media. Wollenweber *et al.* (1925) even suggested that only comparative study of an isolate on different media can give the idea of complete range and size of spore forms in *Fusarium*. Such small differences do not seem to deserve much importance in taxonomy as to warrant creation or maintenance of separate species.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

As in the genus *Fusarium*, the shape and upto some extent size of conidia in *Colletotrichum* also appears to be the most important criterion for delineation of species.

The fungus causing anthracnose disease exhibited a specific choice of host and did not attack even different species of *Dioscorea* other than *D. alata*. Some workers have laid much emphasis on pathogenicity and cross inoculation test in differentiating new species in *Colletotrichum* (Lee, 1924 ; Dastur, 1934 ; Hemmi and Kurata, 1935). Even von Arx (1957 b) has recognised few species only due to their great specificity of host. Ramakrishnan (1941 and 1947) suggested that pathogenic capacity alone should not be given much importance in distinguishing a species as saltants of *C. capsici* differed in their power of infection and morphological characters. He condemned the idea of host selectivity and attributed it to the adaptability of the pathogen. Thus with the knowledge of polyphagic fungi (Berger, 1921) and cross inoculation tests, the criterion of creating new species on different host loses its value as such. However, authors agree with the views of Small (1926) and von Arx (1957) for creation of forms within the species for those pathogenic strains which show a certain degree of selective parasitism and the saprophytic ones recognised as such.

Goto (1929) selected the name *Gloeosporium pestis* Massee for the pathogen attacking *D. alata*, in comparison to *Colletotrichum dioscoreae* Averna, *C. falcatum* Went., *G. amplephagum* (Pass) Sacc. and *G. bomplandii* Speg. von Arx (1957) considered *G. pestis*, *G. bomplandii* and *C. dioscoreae* as synonyms of *C. gloeosporioides* which in turn was conidial *Glomerella cingulata*.

Considering the great specificity of the fungus to the particular species of the host, authors propose to recognise it as a new form *Colletotrichum gloeosporioides f. alatae* forma nov.

SUMMARY

Physiological experiments conducted to see the effect of media, temperature, light etc. on the cultural characters of *Colletotrichum* proved that size and shape of acervuli, presence or absence, size and shape of appressoria and chlamydospores etc. are unstable characters and hence these cannot be taken as the bases for species differentiation within the genus *Colletotrichum*. Shape and upto some extent size of spores was found to be the constant feature and can be relied upon for species classification under this genus. Host range and cross inoculations seem to be helpful only in creation of forms and strains under *C. gloeosporioides* and thus trinomial system of nomenclature is the only resort to check the ever increasing list of arbitrary species. Inspite of great host specificity of the fungus causing anthracnose disease of *D. alata* authors have preferred to recognise it only as a new form *alatae* under *Colletotrichum gloeosporioides* just to mark its great affinity to this species of *Dioscorea*.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The authors are highly grateful to Dr. J. A. von Arx for supplying some of his original papers and to Shri T. C. Kala, Director of Agriculture, Rajasthan for providing laboratory facilities.

Plant Pathology Section,
Department of Agriculture, Jaipur
Rajasthan.

REFERENCES

*BERGER, O. F. (1921) Variation in *Colletotrichum gloeosporioides*. *J. agric. Res.* **20** : 725-736.

*BIRAGHI, A. J. (1934) Sul significato biologico dei presurati 'appressori' nel genus *Gloeosporium* (On the biological significance of the genus *Gloeosporium*). *Boll. R. Staz. pat. Veg. N. S.* **14** : 202.

DASTUR, J. F. (1934) Cotton anthracnose in Central Provinces. *Indian J. agric. Sci.* **4** : 100-120.

*GOTO, K. (1929) On the black spot disease of *Dioscorea alata* and *D. batatas*. *J. Soc. Trop. Agri.* Formosa, **1**, 3 : 301-313.

*HEMMI, T. AND S. KURATA, (1935) Contribution to the knowledge of anthracnose of plants, II. On *Gloeosporium olivarum* Alm. causing the olive anthracnose. *J. Soc. Trop. Agri.* Taiwan **6** : 573-583.

*LEE, H. A. (1924) A disease of Satsuma and Mandarin orange fruits caused by *Gloeosporium foliicolum* Nisshida. *Philip. J. Sci.* **22** : 603-614.

LING, L. AND K. P. LIN (1944) On the occurrence of *Colletotrichum capsici* in China. *Indian J. agric. Sci.* **14** : 162-167.

PRASAD, N. AND R. D. SINGH (1960) Anthracnose disease of *Dioscorea alata* L. (Yam Eng. *Ratalu* Hind.) *Curr. Sci.* **29** : 66-67.

QUINIONE, S. S. (1958) *Taxonomic studies on some species of* Colletotrichum, Thesis for associateship of I. A. R. I. New Delhi.

RAMAKRISHNAN, T. S. (1941) Studies in the genus *Colletotrichum* I. Saltation in *C. capsici* (Syd.). *Proc. Indian Acad. Sci.* **13** (B) : 60-70.

———— (1947) Studies in the genus *Colletotrichum* III. *Proc. Indian Acad. Sci.* **15** B : 15-27.

SINGH, R. D. (1964) *Studies on anthracnose disease of* Dioscorea alata L. Ph. D. Thesis, University of Rajasthan, Jaipur (Unpublished).

SMALL, W. (1926) On the occurrence of a species of *Colletotrichum*. *Trans. Brit. mycol. Soc.* **11** : 112-137.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

von ARX, J. A. (1957) Die arten der Gattung *Colletotrichum* Cda. *Phytopath. Z.* **29** : 413-468.

———— (1957 a) Revision der zu *Gloeosporium* gestellten pilze. *Verh Akad. West. Amst. Ser.* 2, 5 : 153 pp.

———— (1957 b) Kultur-und-Infektion versuche mit einigen *Colletotrichum* Arten. *Tijdschr. Plziekt.* **63** : 171-190.

WOLLENWEBER, H. W., C. D. SHERBAKOFF, O. A. REINKING, H. JOHANN AND A. A. BAILY (1925) Fundamentals for taxonomic studies of *Fusarium. J. agric. Res.* **30** : 833-843.

*Original not seen

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

HELMINTHOSPORIUM DISEASE OF RICE VIII. BREEDING RESISTANT VARIETIES : SELECTION OF RESISTANT VARIETIES OF EARLY DURATION FROM GENETIC STOCK

S. Y. PADMANABHAN, D. GANGULY AND G. H. CHANDWANI

(Accepted for publication November 12, 1965)

INTRODUCTION : One of the major lines of study at the Central Rice Research Institute is the testing of the Genetic Stocks of rice maintained at the Institute for their reaction to blast and helminthosporium diseases of rice. As there are nearly 4000 rice varieties in the Genetic Stocks, the varieties are taken up in convenient lots of about 500 or so at one time. The varieties selected as resistant or moderately resistant in the tests are sent to the various States Department of Agriculture in India for trials of their performance under local conditions.

The results of the varietal susceptibility tests carried out at Cuttack during 1948-1955 with the first lot of varieties consisting of 538 improved strains of rice, have been presented earlier. (Ganguly and Padmanabhan, 1959). In the above tests, six varieties *viz.*, CH-13, CH-45, T-141, Co-20, T-498-2A, BAM-10 were found to be resistant to Helminthosporiose. In the present contribution results are presented of similar tests carried out from 1954 to 1960 with a group of early duration varieties, maturing within 120 days under Cuttack conditions (21°NL).

MATERIALS AND METHODS : Four hundred and ninety varieties of early duration maturing within 120 days were included in the tests. The material was rapidly screened under artificial infection at the seedling stage during the first three years. The tests were carried out in August and February-March.

The techniques and procedures adopted for bringing about artificial infection, scoring the infection, and classifying the types into groups of relative susceptibility have been described in detail earlier (Ganguly and Padmanabhan 1959 l. c.).

The varieties, showing susceptible reaction in these tests were progressively eliminated and the varieties which emerged as relatively resistant were carried forward for further tests under field conditions.

Field tests : The varieties carried forward from the screening tests were subjected to artificial inoculation at seedling stage in the seed bed as well as at the flowering stage in the field. Artificial infection of the seedlings was carried out in the seed bed, when the seedlings were about one month old.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The varieties were transplanted in observation plots 7.5′×4.5′ replicated twice. Artificial infection was carried out at the flowering stage with spore-cum-mycelial dust for three successive evenings. The inoculated plants were covered with wet cloth curtain, suspended from wooden frames (5′×8′×6′). Observations, on the infection which developed, were taken eight days after inoculation. The varieties which emerged as relatively resistant in the tests in the main season (June-October) were also tested in the second crop season (December-March) on the above lines.

RESULTS : Out of 490 varieties, included in the present study, 147 varieties had been already tested during 1948-1955 and eliminated as susceptible. The remaining 343 varieties are classified on the basis of their reaction in the infection tests as follows :—

TABLE 1 : Showing the number of rice varieties tested and their classification in regard to their susceptibility to helminthosporiose.

Kind of test	No. of varieties moderately susceptible to susceptible	No. of varieties moderately resistant.	No. of varieties Resistant.	No. of varieties unclassified.
A. Artificial inoculation tests at Seedling stage, in pots (1954-1956).	319	—	—	—
B. Field tests. (1957-1960).	7	3	4	10*

*These ten varieties out of 343 types could not be classified because the same were not tested sufficiently and they will be included in the next series.

The four resistant and three moderately resistant varieties, selected finally, are mentioned below in Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : List of rice varieties selected finally as resistant or moderately resistant to Helminthosporiose.

A. C. No.	Type No.	Name of the variety	Place of origin	Reaction
1. 1351	T-988	—	China	R*
2. 1382	T-1025	—	—do—	MR*
3. 2041	T-2114	—	—do—	R
4. 2045	T-2118	—	—do—	R
5. 2052	T-2125	—	—do—	MR
6. 2465	HR-21	Hyderabad 21	Andhra Pradesh	MR
7. 2559	T-960	—	Coimbatore Madras	R

**MR—Moderately Resistant.
*R—Resistant.

TABLE 3 : List of varieties which are classified as Moderately Susceptible to *Helminthosporium* disease amongst the early duration varieties (120 days).

Place of origin.	Moderately Susceptible.
1. Assam.	T. 2057/1 (Assam 20/1).
2. Kerala.	T. 253 (Peta chornali).
3. Madras.	T. 540 (Extracted), T. 1488 (Anaikomban), T. 1500 (Kuruva Kalyain), T. 1738 (Swarnawari), T. 2107, T. 2108, T. 991, T. 1015.
4. Mysore.	T. 25 (Ganga Salabatha).
5. Orissa.	T. 945 (Black Mawhri).
6. Punjab.	T. 763 (Karnal, 1064-Pusa).
7. Uttar Pradesh.	H. 287/1, Sur Kharcha, H. 262/1, S. 120 (Bhanli).
8. Australia.	Selus. 44
9. China.	T. 1024
10. Czechoslovakia.	Dhunganshaly.
11. Japan.	T. 1985 (Jap. 11), T. 1986 (Jap. 12), T. 1993 (Jap. 19), T. 1998 (Jap. 6).
12. Russia.	T. 755 (V. Dubanlegaris). T. 918.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SUMMARY

Four hundred ninety varieties of early duration and maturing in 120 days were tested for their resistance to helminthosporiose.

On the basis of their reaction, in the seedling stage under artificial and natural infection, 326 varieties were eliminated as susceptible.

Seven varieties have been selected 4 as Resistant and 3 Moderately Resistant (3) on the basis of their reaction in the seedling as well as adult stages under artificial and natural infection.

Ten varieties have not been sufficiently tested and have not been classified finally.

ACKNOWLEDGEMENT : The authors wish to acknowledge the help rendered by the field staff of Mycology Section in carrying out the pot and field experiments and they are grateful to the Director, Dr. R. H. Richharia, for his suggestions in getting the manuscript ready for publication.

REFERENCES

GANGULY, D. AND S. Y. PADMANABHAN (1959) Helminthosporium disease of Rice-III Breeding Resistant varieties-Selection of Resistant varieties from Genetic Stock. *Indian Phytopath.* **12** : 99-110.

PADMANABHAN S. Y. AND D. GANGULY (1959) Breeding Rice Varieties Resistant to blast disease caused by *Piricularia Oryzae* Cav. 1. Selection of Resistant varieties from the Genetic Stock. *Proc. Indian Acad. Sci.* **L** : (5) Sec. B : 289-304.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SPORE DISPERSAL IN PIRICULARIA ORYZAE CAV.

A. RAMALINGAM

(Accepted for publication January 15, 1966)

INTRODUCTION : *Piricularia oryzae* Cav. the casual organism of the 'blast' disease of rice, is considered to be the most destructive pathogen of rice crop in India. It had been responsible for several epiphytotics in the country (Padmanabhan, 1959). The disease is air-borne and its incidence is mostly influenced by the weather of the season and the phase of the susceptible crop (Padmanabhan and Ganguly, 1954). Some studies for forecasting the 'blast' and some other diseases and pests of rice have been carried out in Argentina (Schultz, 1943), Japan (Kawada, 1954, 1963) and were reported to be in progress at the Central Rice Research Institute, Cuttack, India (Padmanabhan and Chandwani, 1960). Apart from the information collected in the above studies the diurnal periodicity of *P. oryzae* conidia in air has been reported by Panzer, Tullis and Arsdel (1957) and their diurnal spore release under controlled conditions was studied by Barksdale and Asai (1961). During the studies on the air-spora of a paddy field near Visakhapatnam in 1960 and 1961 (Ramalingam, 1962), spores of *P. oryzae* were caught on the slides exposed in a Hirst trap and specific counts were taken for the same. The diurnal and seasonal periodicities of the spores caught, the relation between them and the prevailing weather conditions and stage of crop growth are discussed in the present paper.

MATERIALS AND METHODS : An automatic volumetric spore trap, Hirst trap; (Hirst, 1952) with its orifice 1. 75 metres above the ground level in the centre of an eight acre rice field, near Visakhapatnam, was used to sample the air. Details of the methods followed in this survey were described in full by Sreeramulu and Ramalingam (1965).

A scanning width of 62μ under the oil immersion objective and 12.5x eye-piece was selected for observing and counting the conidia of *P. oryzae* from the slides. The counts were taken at distances corresponding to 2--hourly intervals and were converted into a number per cubic metre of air, taking the efficiency of the trap as sixty per cent.

During the period of this study, rice varieties MTU-19, GEB-24, SLO-16 and PLA-1 were cultivated in the main and second crop seasons (July-December and January-May) respectively.

RESULTS : *Diurnal periodicity and effect of weather* : In order to show their diurnal periodicity in the main and second crop seasons, the mean diurnal curves of the spores caught were constructed separately and are shown in Fig. 1, along with the mean diurnal variations in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

temperature and relative humidity in the respective seasons. The curves show that peak concentrations appeared at 04. 00 hr. (Indian Standard Time) in the main crop season, whereas they appeared at 06. 00 hr. in the second crop season. The concentrations of *P. oryzae* conidia diminished from air more quickly by day in the second crop season than in the main crop season. Peak mean concentrations in both the crop seasons are associated with temperature in the range 22 to 25°C., and relative humidity 85 to 90 per cent (mean values of the seasons). To find out the correct relation between the spore release as understood by spore trap catches and weather conditions, an analysis of weather data on over hundred occasions when the concentrations of *P. oryzae* were higher was made. It has shown that peak concentrations were recorded during nights when the temperature was in the range of 25-27° C., relative humidity 86-98 percent and winds were calm. Rainfall when it occurred during late night hours has put an end to the dispersal of the 'blast' conidia in air.

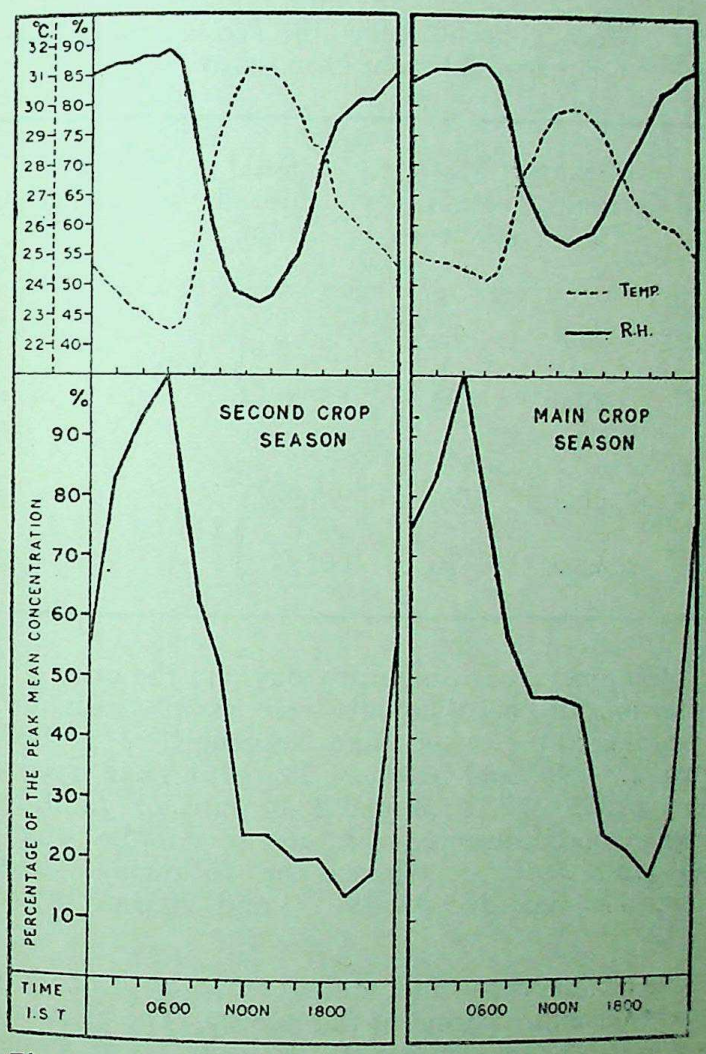


Fig. 1. Mean diurnal periodicity of *Piricularia oryzae* conidia in air during the second and main crop seasons with mean diurnal variations in temperature and relative humidity.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Seasonal periodicity and effect of weather : To show the day to day variations, the daily mean concentrations of *P. oryzae* conidia in air are presented on a time scale in Fig. 2. Air-borne conidia of the fungus showed a marked seasonal periodicity. They appeared in high concentrations in two periods (in each of the two years), one during the period middle of February to middle of April in the second crop season and the other during September to October in the main crop season. These two periods correspond with the periods when the rice crop in the fields was passing through maximum tillering to heading phases and the grasses were in the maximum vegetative growth. The concentrations were higher in the main crop season and low in the second crop season. Data on the relation of temperature, relative humidity and rainfall to the incidence of *P. oryzae* in different seasons, are presented in Table 1. The data show that the mean concentration. of the conidia in the main crop season of 1961 is more than twice that of 1960, which can be related to the high relative humidity of the season as a result of heavy rainfall.

TABLE 1 : Mean concentrations of *Piricularia oryzae* conidia and weather in different crop seasons.

Year	crop season	Mean Temp. °C.	Mean R. H. per cent	Total rainfall mm.		Mean concentration No./m.³ of air	
1960	Second	26.3	73	5.2	560.5	6	8
	Main	27.3	73	555.3		13	
1961	Second	26.8	70	95.9	1110.2	6	16
	Main	26.3	76	1005.3		35	

On rainless nights following rainy days (in the crop growing period) high concentrations of 'blast' conidia were usually caught. For example certain days in the crop seasons like September 31, 1960; October 17, 1960; August 22, 1961 and October 28, 1961 have recorded the daily total amounts of 1.2, 4.7, 21.0 and 21.8 mm. of rainfall respectively. The daily mean concentrations of *P. oryzae* conidia of these days were 0, 33, 7 and 125/m.³ of air, whereas the following days with normal weather conditions, recorded 13, 59, 53 and 205/m.³ of air.

P. oryzae conidia constituted only 0.13 per cent of the total numbers of air spores in the entire period of this survey. The highest concentration recorded was 1,380/m.³ on October 10,1961 at 02.00 hr. Even at this time they constituted only 1.9 per cent of the total number of the air-spora.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

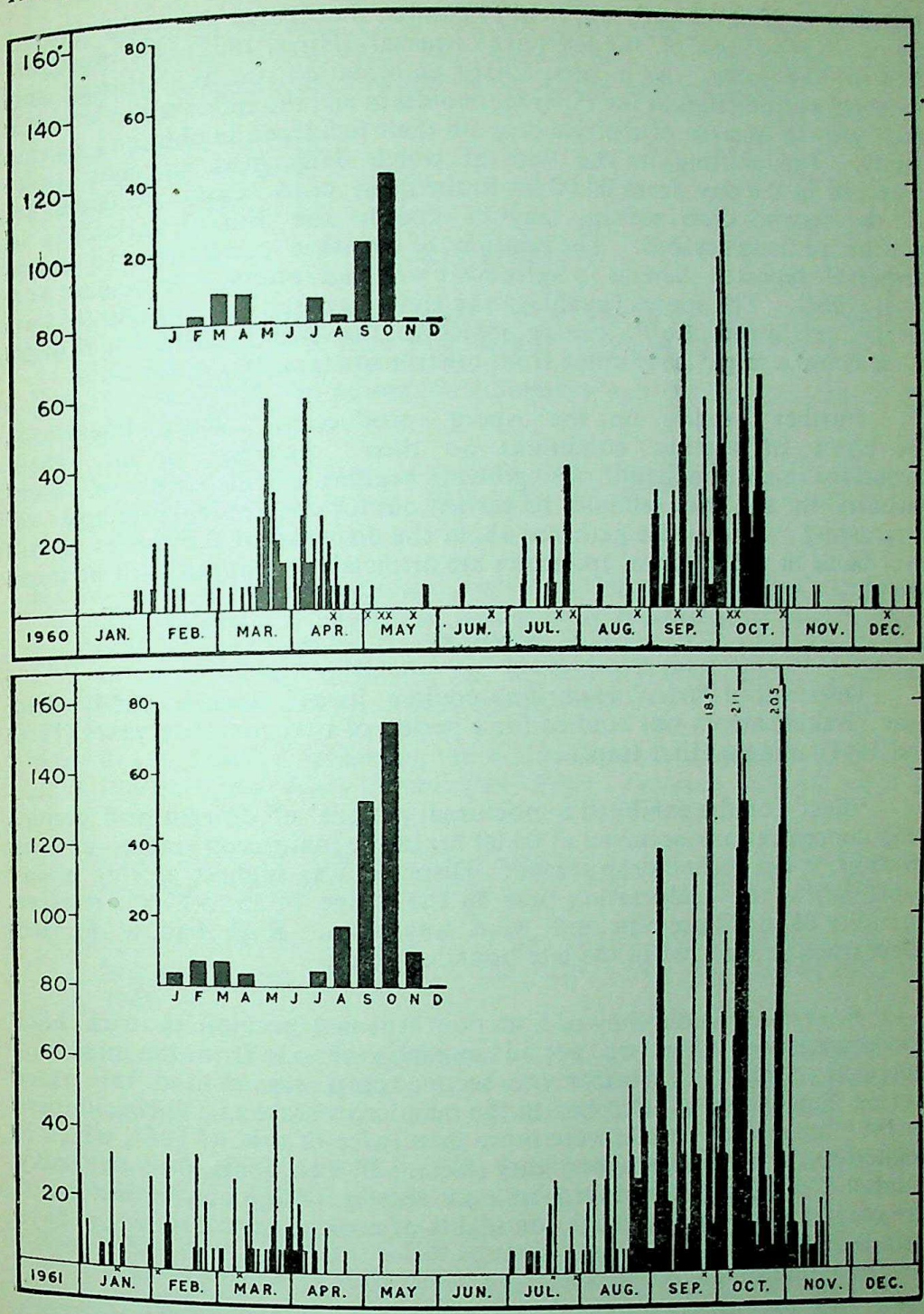


Fig. 2. Daily mean concentrations of 'blast' conidia (number per cubic metre of air) during the years 1960 and 1961. The cross marks indicate the periods on which the data is lost. Monthly mean concentrations are shown as insets.

DISCUSSION AND CONCLUSION : 'Blast' being the most important disease of the rice crop in India, a system of forecasting the outbreak of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the disease, if developed, will help to combat the losses in yield caused by it. Knowledge of the 'effective dispersal' (Hirst, 1959) is a prerequisite for the same. As a preliminary information on the diurnal and seasonal periodicities of the *P.oryzae* conidia in air, the influence of weather and growth phases of the rice crop on their incidence is obtained in this study. The shifting in the time at which daily peak concentrations recurred in the day, from 04.00 hr. in the main crop season to 06.00 hr. in the second crop season, may be due to the diurnal variations in weather in these seasons. The relation of weather conditions to spore dispersal reported here is in agreement with the results of Barksdale and Asai (1961). The spores caught on the slides exposed in the Hirst trap clearly resembled the *P. oryzae* infecting the rice in the field, though some conidia might have come from other hosts (grasses) in the field.

Further studies on the spore production, spore liberation, the effect of weather conditions on their dispersal in air, their variations in air in different rice growing regions of the country, their viability in air, etc., should be carried out for developing principles of forecasting. Knowledge gathered about the dispersal of the spores in air over fields in which 'blast' epidemics are artificially produced, will be most useful.

SUMMARY

Dispersal of *Piricularia oryzae* conidia in air over a paddy field near Visakhapatnam was studied for a period of two complete years (1960 and 1961) using a Hirst trap.

'Blast' conidia exhibited a 'nocturnal pattern' of diurnal periodicity. Peak concentrations occurred at 04.00 hr. in the main crop season and at 06.00 hr. in the second crop season. Dispersal was highest during night hours when the temperature was in the range of 25 to 27°C.; relative humidity 86 to 98 per cent and wind was calm. Rain had a decided effect when it occurred in the late hours of night.

P. oryzae conidia showed a marked seasonal periodicity with high concentrations during two periods in each year, one from the middle of February to middle of April in the second crop season and the other during September to October in the main crop season. Their numbers in 1961 main crop season were more than twice of that of 1960, which is related to the high relative humidity (mean : 76 per cent) due to heavy rainfall (1005.3 mm.) received in that season. High concentrations of the conidia were usually caught on nights of normal days following days with rainfall.

They constituted 0.13 per cent of the total numbers of the air-spora in the entire period of this survey.

The importance of further studies on the 'effective dispersal' of the pathogen to develop principles for forecasting the outbreaks of the disease is emphasized.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : My sincere thanks are due to Dr. T. Sreeramulu for guidance and help. The financial help received from the Indian Council of Agricultural Research is gratefully acknowledged.

Department of Botany,
University of Mysore,
Mysore—6, India.

REFERENCES

BARKSDALE, T. H. AND G. N. ASAI (1961) The diurnal spore release of *Piricularia oryzae* from rice leaves. *Phytopathology* **51** : 313-317.

HIRST, J. M. (1952) An automatic volumetric spore trap. *Ann. appl. Biol.* **39**: 257-265.

HIRST J. M. (1959) Spore liberation and dispersal. *Plant Pathology Problems and Progress* 1908-1958, *ed. Holten et al.*, Madison, pp. 529-538.

KAWADA, A. (1954) The critical basis and present status of forecasting the outbreak of injurious insects and diseases on rice in Japan. *Report for the Fifth Meeting of the Working Party on Rice Breeding October*, 1954. Ministry of Agriculture and Forestry, Japanese Government, Tokyo, Japan, pp. 291-308.

KAWADA A. (1963) Forecasting the injurious insects and diseases in Japan. *International Rice Commission News Letter, Special issue* pp. 127-134.

PADMANABHAN, S. Y. (1959) The present position and control of rice diseases in India. *Proc. Indian Acad. Sci., Sec. B*, **49** : 349-362.

PADMANABHAN, S. Y. AND G. H. CHANDWANI (1960) Forecasting outbreaks of rice diseases in the field. *Rice News Teller*, October, 1960.

PADMANABHAN, S. Y. AND D. GANGULY (1954) Relation between the age of the rice plant and its susceptibility to helminthosporiose and blast diseases. *Proc. Indian Acad. Sci. Sec. B.* **39** : 44-50.

PANZER, J., E. C. TULLIS AND E. P. VAN ARSDEL (1957) A simple 24-hour slide spore collector. *Phytopathology* **47** : 512-514.

RAMALINGAM, A. (1962) *Studies on the air-spora of a paddy field near Visakhapatnam in* 1960 *and* 1961. Doctoral thesis, Andhra University, Waltair, pp. 160.

SCHULTZ, E. F. (1943) *Circ. Estac. Exp. Agric. Tucman* 118 (quoted from *Naha, P. M.* 1961 ; *Nature* **194** : 1061-1063).

SREERAMULU, T. AND A. RAMALINGAM (1966) A two year survey of the air-spora of a paddy field near Visakhapatnam. *Indian J. Agric. Sci.*, (in press)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

WHITE ROOT-ROT DISEASE OF APPLE IN HIMACHAL PRADESH

R. K. AGARWALA AND V. C. SHARMA

(Accepted for publication November 3, 1965)

INTRODUCTION: The root-rot disease of apple trees has been gaining importance in Himachal Pradesh for the past 7-8 years. Agarwala (1961) has reported the association of *Dematophora necatrix* R. Hart. with root-rot of apple.

No work on root-rot of apple has been reported since then from the country or abroad. The earlier work has been reviewed by the senior author in 1961.

The present paper deals with the study on incidence, distribution, predisposing factors, longevity of inoculum and host parasite relationship.

Survey: Sample survey work was taken up in the main apple belts of Mashobra, Mandi, Kotkhai and Kotgarh during 1961-63. 765 orchards comprising of 1,23,060 apple trees and 88 nurseries were surveyed for determining the incidence of white root-rot in differnt apple zones of Himachal Pradesh, and it was observed that the incidence of white root-rot is greater in Mandi (Kulu Valley) than in any other apple belt. Maximum disease incidence on individual orchard basis, however, varied between 12-25 per cent in Orchards at Saroga (Kotgarh). In general, the incidence in apple nurseries ranged between 8-33 per cent.

No orchard has been found completely affected with the disease in any of the apple belt, but hardly any one could also be found completely free from the ravages of this disease. The incidence of the disease in apple orchards located in the elevation range of 1,000 to 2,500 meters above sea level has been classified for determining the elevation range favourable for spread of the disease. The root-rot incidence is only 1.0 per cent upto 1,500 meters but increases with the increase in elevation. The highest incidence of white root-rot has been discovered in orchards at Saroga located at the elevation of 2,356 meters.

Soil temperature exercises an important effect upon the occurrence of soil borne diseases. A direct effect of temperature on the development of soil borne diseases has also been suggested by Bliss (1946) in his studies on *Armillaria mellea*. Similarly the greater incidence of white root-rot at higher elevations may be due to prolonged low temperature conditions. The authors (1962) have also reported temperature between 14°-17°C as optimum range very favourable for the profuse development of *D. necatrix*.

ECONOMIC LOSSES: White root-rot disease of apple destroys the infected trees and the nursery plants resulting in great economic losses. Besides, *D. necatrix* being soil borne fungus, creates unfavourable

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

conditions for other apple trees in an infected orchard. Annual economic losses incurred by the disease in Himachal Pradesh during 1963 have, therefore, been calculated for estimating gravity of the problem.

The losses have been estimated on the basis of standard evaluation formula prepared by the Department of Agriculture, Himachal Pradesh for assessment of compensation granted to apple growers in the event of acquisition of their orchard land for Government purposes.

A. *Basic values of apple plants fixed by the Department of Agriculture, Himachal Pradesh.*

1. Non bearing small plants. — Rs. 15,0 each
2. -do- large plants. — Rs. 30.0 each
3. Apple plants in bearing :— Basic value + income for the remaining bearing life × 1/4 + value for timber varying from Rs. 5/- to Rs. 10/- per plant.

Schedule for calculating income from apple plants in bearing :—

A—Grade	—	Rs. 100.00	each year
B—Grade	—	Rs. 75.00	-do-
C—Grade	—	Rs. 50.00	-do-

Average bearing life of apple plants 30—35 years.

B. *Values considered for calculating economic losses* :

1. Nursery plants :- The losses have been calculated on actual sale price i.e. Rs. 0.75 per plant.

2. Non bearing apple plants : Small and large combined. Rs. 20.00 each.

3. Apple plants in bearing : The value for timber has not been included for calculating the losses. All trees invaded by white root-rot have been considered as B—Grade trees.

C. *Estimated annual losses for 1963* :

a. *Apple nurseries* :

Approximate No. of plants sold	—	400,000
Approximate No. of plants dead due to root-rot at the rate of 10 per cent	—	40,000
Losses at the rate of Rs. 0.75 per plant.	—	Rs. 300,000

b. *Non bearing apple plants :*

Approximate No. of plants in H. P.	—	4,000,000

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Approximate No. of plants dead due to root-rot at the rate of 2.0 per cent	—	8,000
Losses at the rate of Rs. 20.0 each		Rs. 160,000

c. *Apple plants in bearing* :

Approximate No. of plants in H. P.	—	200,000
Approximate No. of plants dead due to root-rot at the rate of 1.5 per cent	—	3,000
Losses at the rate of Rs. 385 per plant.		Rs. 1,155,000
Total annual losses from a, b and c		Rs. 1,345,000

The annual losses due to white root-rot disease of apple were estimated to be in the order of about Rs. 1.3 million during 1963. The estimates are extremely conservative and based upon the most modest figures. The econmic losses are bound to increase in future, keeping pace with the increased horticultural activity envisaged in the future 5 years plans.

SOIL REACTION : Soil pH of 696 apple orchards surveyed in Kotgarh apple belt was tested with a view to determine the relationship between soil pH and the prevalence of white root-rot. The role of pH has been further correlated with invitro studies taken up in the laboratory. *D. necatrix* (one week growth) was inoculated on Richard's liquid medium already buffered at different pH levels. 25 c. c. of liquid medium was placed in 150 c. c. Pyrex conical flasks, and incubated after inoculation for 21 days at 14°C. The macroscopic and microscopic characters of mycelial growth and dry weight at different pH levels were subsequently recorded. The results of soil pH tests showed that *D. necatrix* thrives in field conditions over wide pH range, but soil pH between 6.1 and 6.5 is most favourable.

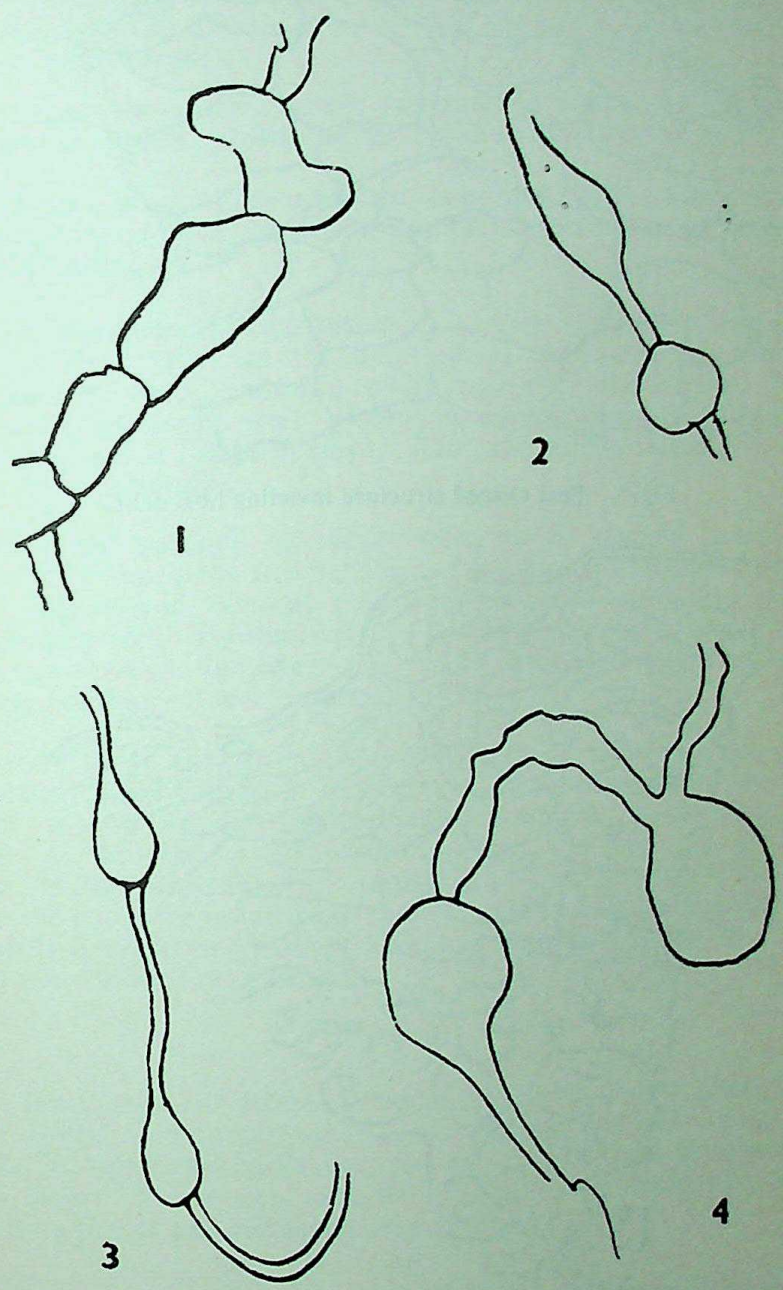
Likewise the maximum fungal growth was at 6.1 pH which decreased on either side of this level in invitro studies. Complete inhibition of mycelial growth did not occur at either of the extreme of pH level tried in the experiment. The swelling of hyphal cells and disappearance of pear shaped structures were, however, very pronounced at the lowest and the highest pH values. The laboratory findings in regard to pH effect on fungal growth were in confirmity with the observation made on the prevalence of the fungus under field conditions.

PLANT AGE : Apple trees in the age-group of 5-20 years have been found to be most susceptible, while infection capacity of the causal organism progressively decreases in trees beyond 20 years of age. The trees are quite hardy against the fungus after 30 years of age.

OTHER FACTORS : Visual observations were undertaken to study the factors contributing to prevalence of white root-rot disease in Himachal Pradesh in 585 orchards and 88 apple nurseries scattered all over the state.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Figs. 1 —4. Influence of different pH levels on mycelial characters of *D necatrix* Fig. 1. Hyphal cells swollen and thickened at 3.0 pH. Fig. 2. Swollen end of the pear shaped structure at 4.1 pH. Fig. 3. Hyphal strands normal with pear shaped swellings at 6.1 pH. Fig. 4. Pear shaped structure very much enlarged at 7.1 pH.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

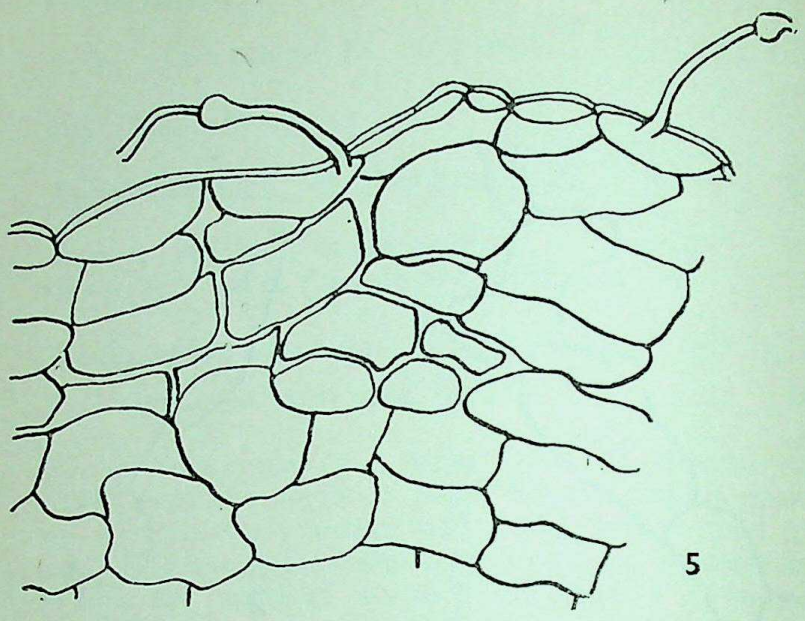


Fig. 5. Pear shaped structure invading host cells

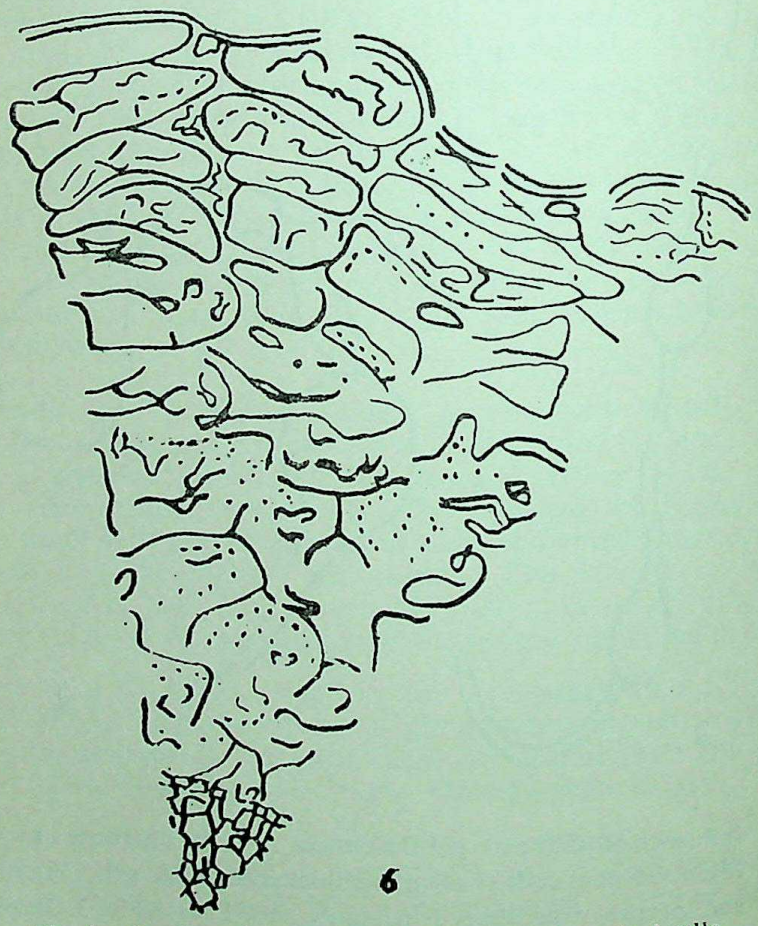


Fig. 6. Section of the damaged phloem and cortical cells.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(i) Mild to severe form of white root-rot incidence was found in about 50 per cent of apple nurseries and the unscrupulous sale of the infected material is a major cause of the spread of the organism to new areas.

(ii) Orchardists, in general, prepare tree to tree channels for drainage of rain water which allows the travel of the infection to healthy trees located on lower terraces.

(iii) High moisture content around the roots was found to be responsible for copious growth of *D. necatrix* on invaded roots.

(iv) Root-rot was more prevalent in new orchards planted in recently cleared forest lands as compared to the older orchards in well established apple belt of Kotgarh.

HOST PARASITE RELATIONSHIP : Studies on the host parasite relationship were taken up for determining the mode of infection of *Dematophora necatrix*, causing white root-rot of apple. Healthy fibrous roots of apple were placed in association with infected apple roots under moist soil kept in trays. The infection started in healthy roots within a fortnight.

Microtome sections of the invaded roots showed pear shaped structures of *D. necatrix* forcing their entry through the epidermal cells (Fig. 5). Phloem and cortical cells were damaged at various points in later stages (Fig. 6). The cavities so created, contained hyphal cells of the organism. Although there was no distortion of xylem cells at any stage, necrosis was evident in some of the cells.

LONGEVITY OF THE PARASITE : Apple roots with copious growth of *D. necatrix* were collected from affected trees during the rainy season alongwith the soil around each disease sample. These samples were stored in the laboratory in separate trays. Occasionally water was sprinkled for the maintenance of moisture. The organism could remain active on the roots for an average period of 345 days. *D. necatrix* was subsequently overgrown by the profuse and dominant growth of *Pythium*, *Rhizopus* and *Penicillium* species.

SUMMARY

1. White root-rot disease of apple caused by *Dematophora necatrix* has been found widely prevalent in Himachal Pradesh. The sample survey of 765 apple orchards comprising 123,060 trees besides 88 apple nurseries gave 1.5 per cent and 9.0 -33 per cent incidence of white root-rot in orchards and nurseries respectively.

2. The maximum incidence of the disease was recorded at elevations ranging between 1,800 to 2,400 meters. The economic losses due to the spread of white root-rot have been estimated of the order of Rs. 1.3 million per year.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

3. The soil pH between 6.1-6.5 was determined as optimum for good growth of the fungus. Plant age in relation to predisposition of the disease and visual observations on several other causative factors have been recorded and discussed.

4. Host parasite relationship of *D. necatrix* has been studied. The organism on invasion, ruptures the cortical and phloem cells which disrupts the translocatory system.

5. The maximum period of the active life of *D. necatrix* in situ was found to be 345 days.

ACKNOWLEDGEMENTS : The authors are greatly indebted to the Indian Council of Agricultural Research and Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh, for providing financial assistance and the laboratory facilities without which it would have been difficult to complete this work.

Regional Fruit Research Station,
Mashobra, Simla-7.

REFERENCES

AGARWALA, R. K. (1961) Problem of root-rot in Himachal Pradesh and prospects of its control with antibiotics. *Himachal Horticulture* **2** : 171-178.

——— — (1962) Annual Progress Report of the Scheme for "Intensification of Research on Root-rot and Collar-rot diseases of apple in Himachal Pradesh."

BLISS, D. E. (1945) The relation of soil temperature to the development of *Armillaria* root-rot. *Phytopathology* **36** : 312-318.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A STUDY OF INTERACTION BETWEEN CEPHALOSPORIUM SP. AND AZOTOBACTER CHROOCOCCUM

N. S. SUBBA-RAO AND V. ISWARAN

(Accepted for publication January 21, 1966)

The nature and frequency of fungi occurring on legume root nodules, their interaction with rhizobia and the probable importance of these fungi in phosphate solubilization have been reported earlier (Subba-Rao and Vasantha, 1965: Subba-Rao and Bajpai, 1965). Screening of culture filtrates of these fungi revealed that a species of *Cephalosporium* inhibited the growth of four strains of *Azotobacter chroococcum* on agar plates. The fungus has also been found frequently in Delhi soils as revealed by the soil dilution plate method. Since *Azotobacter* is known to play a vital role in the nitrogen economy of Indian soils, it was considered appropriate to extend this study. The present report relates to the effect of *Cephalosporium* Sp. on the nitrogen fixing ability of *A. chroococcum* in culture and in pots, using wheat variety N P 780 (*Triticum aestivum* L.) as the test plant.

MATERIAL AND METHODS: *Cephalosporium* Sp. was maintained on potato-dextrose agar slants and *A. chroococcum* on Jensen's agar slants. The fungus was grown on 250 ml. Richard's synthetic liquid medium in conical flasks of 1 liter capacity for 3 weeks. The culture filtrate had a pH of 5.9. It was later adjusted to pH 7.0 and concentrated to 20 ml. at 40-45°C. Filter paper discs were soaked in the culture filtrate, dried and placed on Jensen's agar in Petri plates seeded with *Azotobacter*; formation of clear zones around the filter paper discs after 24 hours incubation at 32°C indicated the antibiotic activity of the fungal filtrate. The average diameter of inhibition zones around four filter paper discs were recorded for each strain of the bacterium. Four different strains of *Azotobacter* (from Russia, Samalkot, Delhi and Cuttack) were used for *in vitro* experiments.

To obtain quantitative data regarding the extent to which *Cephalosporium* Sp. altered the nitrogen fixing ability of *Azotobacter*, the fungus and each strain of the bacterium were simultaneously inoculated into 25 ml. Jensen's liquid medium in 250 ml. conical flasks. The flasks were incubated at 32°C for 2 weeks at the end of which nitrogen estimation of the contents of the flasks was done.

In the green house test, earthenware pots of 10 lb. capacity containing washed quartz sand was used. Fungal mat (100 g. fresh weight) obtained from 3-week old culture of *Cephalosporium* Sp. grown on Richard's synthetic substrate was washed in sterile water and gently macerated in a waring blendor. The resulting suspension was poured in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

equal quantities to pots with sterile distilled water. The sand was mixed with the inoculum to a depth of 4 inches and wheat seed was sown at the rate of 10 seed per pot. As soon as the seed germinated, equal aliquots of a suspension of *A. chroococcum*, Delhi strain, were added uniformly to pots around the roots of seedlings. The plants were watered when necessary and nitrate-free nutrient solution was added once a week. The nutrient solution used was that of Crone, modified by Bond (Allen, 1959). The number of seedlings was thinned to four at the end of 21 days. The pots were incubated in the green house for 4 months at the end of which fresh and dry weights of plants and their nitrogen content were determined. Nitrogen was estimated by micro-Kjeldahl method.

RESULTS : The average diameter of inhibition zones on *Azotobacter*-seeded nutrient agar plates for the Russian, Cuttack, Samalkot and Delhi strains were respectively 25.00 mm., 24.00 mm., 25.00 mm. and 22.00 mm. Result of *in vitro* study on the nitrogen fixing ability of *A. chroococcum* in the presence of *Cephalosporium* Sp. is presented in Table 1. Generally speaking, nitrogen fixation by *Azotobacter* was significantly inhibited in the presence of *Cephalosporium* Sp. It was apparent that this inhibition was seen to the maximum in the Cuttack strain where 42.42 per cent decrease over control in the amount of nitrogen fixed occurred. The minimum inhibition occurred in the Russian strain of *Azotobacter* where a decrease in the amount of nitrogen fixed took place to the extent of nearly 21.91 per cent while the decrease in the Samalkot and Delhi strains was of the order of 29.12 per cent and 36.12 per cent respectively, over the corresponding controls. An examination of the fresh weight, dry weight and nitrogen status of plants in the seedling stage (Table 2) revealed that while no appreciable difference in the weight among the different treatments was noticeable, a decrease in the nitrogen content of the plant tissue was, however, characteristic of *Azotobacter* plus *Cephalosporium* treatment when compared to *Azotobacter* treatment ; this trend towards decrease in the nitrogen content of the plant tissue was further apparent from the data on the nitrogen status of grain and straw at harvest (Table 3). When the nitrogen status of control plants at harvest was compared with that of plants treated with *Cephalosporium* Sp., it was obvious that the latter showed a decrease in the nitrogen content.

Judging from the yields of straw and grain from pot trial (Table 3), it could be concluded that myco-colonization of *Cephalosporium* in sand had a significant effect in minimizing the beneficial effect of *Azotobacter* inoculation. From the point of view of yield of straw and grain, a decrease in the yield was characteristic of plants treated with *Cephalosporium* plus *Azotobacter* when compared with the plants treated with *Azotobacter*. Similarly, when the yield of control plants was compared with that of plants treated with *Cephalosporium* Sp., a decrease in both grain and straw in the latter became evident.

DISCUSSION : Based on the yields of straw and grain and the nitrogen status of plants in the four treatments in the pot trial, it could be concluded that *Azotobacter* inoculation in sand improved the nitrogen

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and yield status of wheat plants. However, myco-colonization of *Cephalosporium* Sp. in sand had a significant effect in minimizing the beneficial effect of *Azotobacter* inoculation. Although no toxic symptoms were noticeable in plants treated with *Cephalosporium*, the decrease in the nitrogen content of plants when compared with uninoculated controls may be due to the interference of fungal metabolites in protein synthesis and nitrogen metabolism.

TABLE 1 : Effect of *Cephalosporium* Sp. on the nitrogen fixation by four strains of *Azotobacter chroococcum in vitro*.

Treatment		Mg. N fixed/ 25 ml. medium (average of 4 replications)	Per cent Decrease over control
A. chroococcum	Russian strain	8.37	—
A. chroococcum,	Russian strain plus *Cephalosporium* Sp.	6.68	21.91
A. chroococcum,	Samalkot strain	7.21	—
A. chroococcum,	Samalkot strain plus *Cephalosporium* Sp.	5.11	29.12
A. chroococcum,	Delhi strain	6.45	—
A. chroococcum,	Delhi strain plus *Cephalosporium* Sp.	4.22	36.12
A. chroococcum,	Cuttack strain	6.69	—
A. chroococcum,	Cuttack strain plus *Cephalosporium* Sp.	3.99	42.42

C. D. at 5 per cent level = 0.81.

TABLE 2 : Effect of combined inoculation of *Cephalosporium* Sp. and *A. chroococcum*, Delhi strain on wheat, at seedling stage.

Treatment	No. of plants	(21 days old seedlings) Weight per plant (mg.)		Percentage nitrogen
		Fresh	Dry	
Control	32	9.5	1.65	1.21
A. chroococcum	29	10-5	1.68	1.99
A. chroococcum + *Cephalosporium* Sp.	30	10.5	1.67	1.53
Cephalosporium Sp.	29	10.7	1.71	1.24

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 3 : Effect of combined inoculation of *Cephalosporium* Sp. and *A. chroococcum* on wheat crop at harvest (4 months old plants).

Treatment	Yield in g./pot*		Nitrogen in g./pot*		Total nitrogen in grain and straw per pot*	Percentage nitrogen		Gain of nitrogen over control due to inoculation
	Grain	Straw	Grain	Straw		Grain	Straw	
Control	3.75	6.00	0.074	0.020	0.094	1.93	0.42	—
A. chroococcum	6.70	8.20	0.138	0.052	0.190	2.06	0.64	+0.096
A. chroococcum+ *Cephalosporium* Sp.	5.00	6.80	0.076	0.025	0.101	1.53	0.38	+0.007
Cephalosporium Sp.	3.05	5.20	0.041	0.016	0.057	1.36	0.31	−0.037

C. D. at 5 per cent level for yield of grain=0.30.
C. D. at 5 per cent level for yield of straw=0.27.
*Average of 5 replicates.

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Results of *in vitro* studies on the effect of *Cephalosporium* Sp. on the nitrogen fixing ability of different strains of *A. chroococcum* showed that the Cuttack strain was the most sensitive one and the Russian strain, the least sensitive ; the Samalkot and Delhi strains fall in between the Cuttack and Russian strains.

Antibiosis in soil by antibiotics produced by micro-organisms is now well recognised. Studies on nitrogen fixation by *Azotobacter* in association with bacteria and actinomycetes (Gadgil and Bhide, 1960) showed that some actinomycetes stimulated nitrogen fixation while others depressed it. The present report demonstrates the occurrence of inhibitory activity of *Cephalosporium* Sp. towards nitrogen fixation by *Azotobacter* which indeed reflects the desirability of investigating the several interactions of diverse soil fungi with free living as well as symbiotic nitrogen fixing micro-organisms. The implications of such studies lie not only in the obvious necessity for developing suitable strains of nitrogen fixing bacteria resistant to antibiotics but also in evaluating the causative factors in the success or failure of an introduced organism into a given soil.

Summary

Growth and nitrogen fixation of *Azotobacter chroococcum* was inhibited by a species of *Cephalosporium*. In pot trial, judging from the yields of straw and grain and nitrogen content of plant tissue, myco-colonization of *Cephalosporium* Sp. in sand had a significant effect in minimizing the beneficial effect of *Azotobacter* inoculations of wheat plants.

Acknowledgement : The authors are thankful to Dr. W. V. B. Sundara Rao, Head of the Division of Microbiology for his interest in the investigation.

Division of Microbiology,
Indian Agricultural Research Institute,
New Delhi.

References

Allen, O. N. (1959) *Experiments in Soil bacteriology*, 93 pp., Burgess Publishing Co., Minnesota.

Gadgil, P. O. and V. P. Bhide (1960) Nitrogen fixation by *Azotobacter* in association with some associated soil microorganisms. *Proc. Nat. Inst. Sci. India.* **26** : 60-63.

Subba-Rao, N. S. and P. D. Bajpai (1965) Fungi on the surface of legume root nodules and phosphate solubilization. *Experientia* **21** : 386—387.

Subba-Rao, N. S. and P. Vasantha (1965) Fungi on the nodular surface of some legumes. *Naturwissenschaften* **52** : 44.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BIOCHEMISTRY OF FUSARIUM WILT RESISTANCE IN COTTON

I. THE INFLUENCE OF AMINO ACIDS ON FUNGAL GROWTH

A. MAHADEVAN

(Accepted for publication, January 25, 1966)

The disease resistance mechanism in cotton plant against *Fusarium oxysporum* f. *vasinfectum* (Atk.,) Snyder and Hansen is little understood, although previous investigators (Lakshminarayanan, 1955 and Subramanian, 1956) suggested the possible mechanisms of resistance. Lakshminarayanan (1955) suggested that by limiting the quantity of pectin, the resistant plants discouraged the pathogen from secreting the enzymes polygalacturonase and pectin methyl esterase which are responsible for pathogenicity. He also showed that cystine and other sulphur containing amino acids were associated with disease resistance and demonstrated that cystine and glutamic acid offered effective protection to the cut shoots of cotton against the *in vitro* toxaemia. Subsequently, Subramanian (1956) also suggested that cystine may play a part in wilt resistance.

With a view to examine the influence of cystine, cysteine and glutamic acid on the growth of *F. oxysporum* f. *vasinfectum*, the present studies were carried out.

MATERIALS AND METHODS : The culture used in the present studies, *F. oxysporum* f. *vasinfectum* was obtained from the University Botany Laboratory, Madras-5.

Fifty ml. portions of Czapek's medium at pH 6.5 containing the test compounds (cystine, cysteine and glutamic acid) at 0.1 and 0.25 per cent concentrations were dispensed in 250 ml. Erlenmeyer flasks and sterilized. The flasks were inoculated with a 8 mm. disc of the fungal culture obtained from Czapek's agar medium and were incubated at laboratory temperature (28±2°C).

To examine the additive or synergistic effects of the amino acids on fungal growth, all the three amino acids were added in different combinations to reach the final concentrations of either 0.1 or 0.25 per cent. The media were inoculated and the cultures were incubated upto 20 days. The mycelium was separated from the medium on a previously dried and weighed filter paper and the dry weight of the fungal mat was determined after drying in an oven at 102°C to obtain a constant weight. The tests were carried out in triplicates.

RESULTS : The inhibitory action of the amino acids on fungal growth is shown in Table 1. All the three amino acids inhibited fungal growth, the inhibition being lesser at the higher concentrations 0.25 per-

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

cent, except cysteine which inhibited more at 0.25 per cent. A combination of cysteine-cystine was more inhibitory when compared with the other two combinations at 0.1 per cent concentrations. In the combinations of cysteine-glutamic acid and cystine-glutamic acid, the inhibition was more at 0.25 per cent concentration than at 0.1 per cent. When all the three amino acids were combined, the inhibition was more at the lower concentration.

TABLE : The influence of amino acids on the growth of *Fusarium oxysporum* f. *vasinfectum*

Amino acid (s)	Dry wt. of mycelium. per cent over control*	
	Concentration of amino acids 0.1 per cent	0.25 per cent
Cystine	75	94
Cysteine	60	43
Glutamic acid	63	90
Cystine-Cysteine	60	90
Cysteine-Glutamic acid	73	60
Cystine-Glutamic acid	75	60
Cystine-Cysteine-Glutamic acid	60	70
Czapek's (no amino acid) Control	100	

*The data represent averages of the triplicates.

DISCUSSION : The results obtained in the present studies show the inhibitory effect of amino acids on the mycelial growth of *F. oxysporum* f. *vasinfectum* at 0.1 and 0.25 per cent concentrations, which are within the physiological concentrations in the cotton plant. The two sulphur containing amino acids, cystine and cysteine, inhibited the fungal development when tested individually as well as in combination. Glutamic acid also inhibited the fungal growth. It is generally assumed that cysteine is oxidized to cystine by cytochrome C oxidase system (Kun, 1961). Probably, cysteine conversion to cystine helped to inhibit the fungus, especially synergistically at lower concentrations. The mode of inhibition by cysteine-cystine on fungal growth requires to be determined. It is well known that glutamic acid enters many biochemical pathways of the fungus. It enters transamination reactions and also takes part in the interconversion of ornithine, proline and hydroxyproline (Greenberg, 1961). *F. oxysporum* f. *vasinfectum* has been shown to utilize glutamic acid as a sole nitrogen source. The mode of inhibition of the fungal growth by glutamic acid needs further investigation.

The influence of cysteine, cystine and glutamic acid on the production of fusaric acid, one of the important toxins elaborated by the wilt fungus (Lakshminarayanan and Subramanian, 1955) requires further

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

studies. The possibility of amino acid-phenol interactions *in vivo* and studies involving such interactions in cotton plant with *F. oxysporum* f. *vasinfectum* infections need elucidation.

SUMMARY

The influence of cystine, cysteine and glutamic acid on the growth of *Fusarium oxysporium* f. *vasinfectum*, the cotton wilt pathogen was studied. All the three amino acids inhibited the fungal growth when tested at 0.1 and 0.25 per cent concentrations in liquid cultures. There were indications for certain amount of additive or synergistic effect of all amino acids in inhibiting the fungal growth. The possible influence of amino acids in disease resistance is discussed.

ACKNOWLEDGEMENT : The author expresses his sincere thanks to Professor G. Rangaswami for his interest during the progress of the work and his critical suggestions during the preparation of the manuscript.

Microbiology Laboratory,
Annamalai University,
Annamalainagar, S. India.

REFERENCES

GREENBERG, D. (1961) Carbon catabolism of amino acids—in Metabolic Pathways, edited by Greenberg, D. Academic Press, N. Y.

KUN, E. (1961) The metabolism of sulphur containing compounds in Metabolic Pathways, edited by Greenberg, D. Academic Press, N. Y.

LAKSHMINARAYANAN, K. (1955) Studies on fungal enzymes with special reference to the *Fusarium* wilt of cotton. Doctoral thesis, Madras University, Madras.

LAKSHMINARAYANAN, K. AND D. SUBRAMANIAN (1955) Is fusaric acid a vivo toxin ? *Nature*. **176** : 697-698.

SUBRAMANIAN, D. (1956) Studies on the control of fungal wilts of plants. Doctoral thesis, Madras University, Madras.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

PATHOLOGICAL AND EPIDEMIOLOGICAL STUDIES ON LEAF BLIGHT OF WHEAT CAUSED BY ALTERNARIA TRITICINA

A. S. PRABHU AND R. PRASADA

(Accepted for publication December 15, 1965)

INTRODUCTION : A very destructive leaf blight disease of wheat has been reported from several States in recent years. The failure of Kenphad wheats which had been evolved in the Maharashtra State for resistance to rust after several years of breeding was attributed to the heavy damage caused by leaf blight disease resulting in their withdrawal from cultivators' fields. Recently this disease came up for discussion in the All-India Wheat Research Workers Seminar (1961) at which various workers from different parts of the country gave alarming reports of its destructive power and emphasized that the research for combating it should be intensified urgently. During the 1963-64 wheat season the outbreak of the disease was so severe at the Botanical Sub-Station, Pusa, Bihar, that not a single genetic stock under cultivation there had escaped the disease (Prasada and Prabhu, 1964).

The occurrence of this disease was first recorded by Kulkarni from Bombay Presidency and McRae from Pusa, Bihar, in 1924 and later by various investigators from different regions of the country (McRae, 1933; Mehta, 1950 ; Mathur, 1956 ; Bose, 1956 ; Bener Raj, 1960). According to them a species of *Alternaria* was associated with the disease. Mathur (1956) however, is reported to have isolated *A. tenuis* auct. from the diseased leaves and reproduced leaf spots on one week old wheat seedlings. It might be mentioned that this disease has not so far been reported from any other part of the world. Although, in India the disease had been noticed much earlier, it did not cause much concern till very recently when it became widespread.

The earlier work mainly related to the mere recording of the disease associated with an unidentified species of *Alternaria* and the pathogenicity of the organism had not been proved conclusively. The present authors in an earlier note, Prasada and Prabhu (1962), have definitely established that the leaf blight is caused by a new species of *Alternaria* and named it as *A. triticina* Prasada and Prabhu. Considering the importance of the disease, and the scanty information available, detailed investigations were taken up in a systematic way and the results are presented in this paper.

MATERIAL AND METHODS : Diseased wheat leaves showing typical symptoms were collected from the fields of Botany Division, Indian Agricultural Research Institute, New Delhi, and the organism isolated from them by plating surface sterilized bits on potato dextrose agar. Also, isolations were made from the diseased leaf material obtained from Pusa

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

and Indore substations of Indian Agricultural Research Institute. The cultures were purified by making single spore isolations. Seeds affected with black point disease were collected and the organism causing the black point was brought into culture. Authentic culture of *Alternaria tenuis* auct. was obtained from Indian Type Culture Collection for comparative studies.

All inoculation experiments for studies on pathogenicity were conducted in a greenhouse. About 4 to 5 week old plants of susceptible wheat varieties, viz. N. P. 827, N. P. 828, N. P. 830, Niphad 4 and Kenphad 21 grown in 10″ pots were used in the tests. The cultures of *A. triticina* were multiplied on standard nutrient agar (K_2HPO_4 1.36 gms. Na_2CO_3 1.06 gms. ; Mg $SO_4$7 H_2O 5.0 gms. ; dextrose 5.0 gms. ; asparagine 1.0 gm ; agar 20.0 gms and water 100 cc) which was earlier determined to be best for sporulation. For purposes of inoculation, a thick conidial suspension was made in water and sprayed on the plants with an atomizer. The inoculated plants were labelled and incubated in humid chamber for a period of 72 hours. They were then transferred to greenhouse benches. Suitable controls were maintained in every experiment.

Inoculations were also made on sterile seedlings in test tubes by the modified method of V. Luijk (1934, 1938) employed by Neergaard (1944). A cylinder of filter paper about an inch in length closed above and open below was pushed in a test tube containing 10 cc. of water in such a way that its closed end remains just above the water level inside. The tubes were plugged with non-absorbent cotton and autoclaved at 15 lbs. pressure for 20 minutes. Surface sterilized seeds were placed on the flat upper surface of the paper cylinder. Inoculations were made after the plants had reached first leaf stage by spraying suspension of the spores with an atomizer under aseptic conditions.

Various relative humidity levels (100 per cent, 98.5 per cent, 96.1 per cent, 92.9 per cent, 88.5 per cent) were obtained in petriplates at 25°C by using different dilutions of C. P. sulphuric acid (McLean and Cook, 1941) to study the effect of humidity on spore germination.

Histological observations were made by clearing and staining unsectioned inoculated leaf bits in 1 per cent lactophenol-cotton blue. Free hand sections were made to study the histological changes brought about by the infection.

EXPERIMENTAL : *Symptoms of the leaf blight disease* : The disease first makes its appearance as small, oval, discoloured lesions, irregularly scattered on the leaves. The spots as they enlarge become irregular in shape and take up dark brown to grey colour. A bright yellow marginal zone is sometimes seen around the spots (Fig. 1) which are of varying dimensions and attain a diameter of 1 cm. or more. As the disease progresses, several lesions coalesce and cover large areas resulting

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

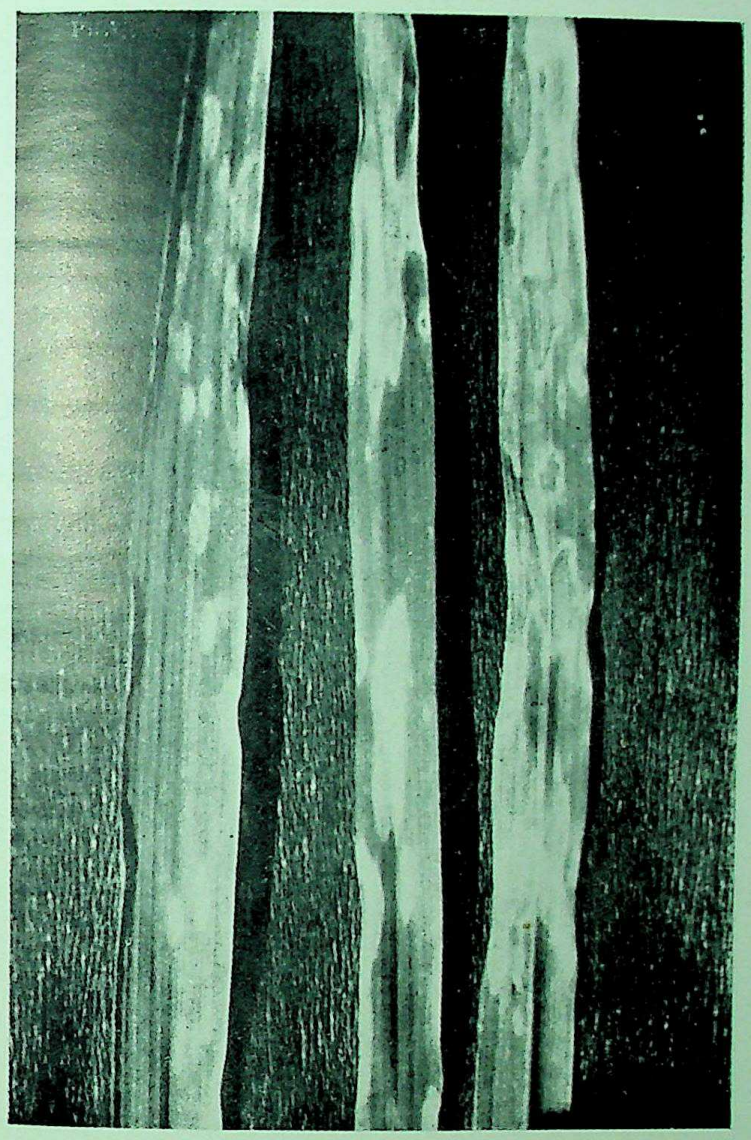


Fig. 1. Symptoms of leaf blight caused by *Alternaria triticina* on wheat leaves.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar.

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 3. Conidiophores bearing conidia of *Alternaria triticina* emerging through the stomata on surface of a wheat leaf.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

in the death of entire leaf. In some cases the leaf starts drying up from the tip prematurely with the appearance of the lesions. Black powdery conidia cover the lesions at this stage under moist conditions.

The lowermost leaves are always the first to show the signs of infection which gradually spreads to the upper leaves. In severe cases of infection, similar symptoms are produced on the leaf sheath, and in the ear, the awns and glumes.

In the field the disease appears when the plants are seven to eight weeks old and becomes virulent in the latter half of February when the crop is mature. Starting along the irrigation canals where the soil moisture is high, it spreads rapidly until the whole field is involved. Heavily infected fields present a burnt appearance and can be made out from a distance and since this is the most conspicuous phase of the disease, it has been called as 'leaf blight'.

Isolation and proof of pathogenicity : A large number of isolations made in the month of February 1960 from the diseased leaves of wheat varieties N. P. 828 and N. P. 830 collected from Delhi yielded a pure culture of *Alternaria* sp. which was later identified as a new species of *Alternaria* and named as *A. triticina* (Prasada and Prabhu, 1962). In some isolations however, *A. tenuis* was also encountered.

Repeated inoculations were made on one week old seedlings and one month old plants of wheat varieties N. P. 828, N. P. 830, Niphad and Kenphad with the isolates of *A. triticina* and *A. tenuis*, simultaneously. In all the tests young seedlings failed to get infected whereas one-month old plants of all the four varieties were heavily infected but only with *A. triticina*.

Reisolates from the infected spots resembled the original culture of *A. triticina*. Single spore isolations were made from these cultures and the pathogenicity repeatedly proved on susceptible varieties mentioned above. One such culture was selected for detailed study.

Subsequently, pathogenicity of *Alternaria* sp. isolated from the diseased leaf material obtained from a large number of collections from U. P., Bihar and M. P. was proved.

Experimental evidence has been obtained which shows that *A. tenuis* isolated from the infected wheat leaves does not produce any disease symptoms either in the seedling or the older plants, Similar results were obtained with *A. tenuis* isolated from the black point affected wheat seed showing thereby that this organism is restricted to the seeds and does not cause the leaf blight.

Infection experiment on seedlings : In order to confirm the above evidence that only adult plants are susceptible, young seedlings were inoculated with *A. triticina* in test tubes under aseptic conditions as described in Material and Methods. This method was characterized by

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Neergaard (1945) as very sensitive and even a faint degree of parasitism will manifest itself. Inoculations were performed by atomizing the spore suspension made in sterile water. Three isolates of *A. triticina*, one each from Delhi, Pusa and Indore, the pathogenicity of which on the adult plants of wheat had been proved in our earlier experiments, were used in inoculating twelve seedlings of each of the four susceptible varieties N. P. 828, N. P. 830, Niphad 4 and Kenphad 21. The experiment was repeated thrice and in all instances the seedlings were as healthy as in uninoculated controls, showing thereby that the pathogene is unable to infect young seedlings.

Relation of age to susceptibility : The inoculation experiments conducted earlier had proved that vigorously growing young seedlings offer resistance to *A. triticina*. In order to find out the stage of growth of the host plants at which the resistance breaks down, plants of different ages were inoculated, simultaneously, by spraying the spore suspension. The susceptible variety Kenphad was used in this experiment. The age of the plants was calculated from the date of sowing. The results are presented in Table I.

TABLE 1 : Results of inoculations on plants of different ages with *Alternaria triticina*.

Age of the plants in days	No. of leaves inoculated	No. of leaves infected	Remarks
7	14	0	First leaf stage; no infection.
15	26	0	Second leaf stage; no infection.
22	39	6	Third leaf stage ; one or two spots on the lowermost leaf.
30	40	18	Fourth leaf stage ; lowermost three leaves showed spots.
38	45	29	Fifth leaf stage; spotting on the lowermost four leaves of each plant.
115	134	84	Boot stage of the plant; severe infection on all the leaves excepting flag leaf.
125	106	97	Ear stage; all the leaves including the flag leaf showed spots.

It can be observed from the data given in Table I that 7 to 15 days old seedlings are not susceptible to infection. Only when the plants had been inoculated at the third leaf stage (22 days old plants) one or two spots were observed on the lowermost leaf. One month old plants and those

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

older than that showed good infection in which all the leaves excepting the top leaf produced leaf spots. In every case the number of spots found on the lowermost leaves was greater than that on the leaves above them. In the boot stage of the plant the infection was severe on all the leaves excepting the boot leaf which remained green and healthy. After the ear had emerged from the boot all the leaves including the flag leaf showed symptoms of the disease. The glumes, awns, leaf sheaths and even the culms were seen infected.

The young vigorously growing top leaf was not infected till the ear was formed. The spots were seen to enlarge rapidly on the fully matured plant and in some cases the leaf, to start with, dried up from the tip downwards with the development of the spots.

Inoculation experiments on the ear : The constant association of *Alternaria tenuis* with the black point affected seed, had led some of the earlier investigators to suspect that it was the causal agent of the leaf blight disease also, and thus the latter was regarded to be seed-borne. It has been shown experimentally in the present work that *A. tenuis* associated with the black point disease of wheat is confined to wheat seed and does not cause leaf blight. Since the glumes and awns have been shown to get infected with *A. triticina* in the greenhouse, it was felt necessary to determine, experimentally, to what extent the black point organism and the leaf blight organism differ in their capacity to attack the glumes, awns and, in turn, the seed. In these experiments, the varieties Niphad and Kenphad were used as they are known to be susceptible to both leaf blight and black point diseases. Inoculations were made separately with *A. triticina* and with *A. tenuis* isolated from the black point affected seed. Plants at different stages of grain formation were inoculated with spore suspension by the following methods :

I. The spore suspension was injected by means of a hypodermic syringe into the leaf sheath enclosing the spike of the plant (boot stage of growth).

II. The spore suspension was placed or injected by means of a dropper into the florets of wheat.

III. A suspension of the spores of the fungus was sprayed on the spikes.

The concentration of the spore suspension in both the organisms was made approximately equal. Care was taken not to contaminate the two cultures with each other. After each inoculation, the hypodermic syringe was washed with alcohol and then with water before use a second time. In the controls, sterile water was injected instead of spore suspension. The results of syringe inoculation of boot are presented in Table 2.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 2 : Comparative results of inoculation of boot with spore suspension of *A. tenuis* causing black point disease and *A. triticina*, the causal organism of leaf blight disease.

Isolate	Niphad variety		Kenphad variety		Remarks
	No. of boots inoculated	No. of boots infected	No. of boots inoculated	No. of boots infected	
A. tenuis	23	0	16	0	Emergence of the ear normal, no chlorotic streaks on the boot leaf
A. triticina	32	32	19	19	No ear emergence excepting in two cases, chlorotic streaks along the mid rib of the boot leaf
Control	10	0	8	0	Emergence of the ear normal

The results presented in Table 2 show that *A. triticina* infected all the inoculated boots. The leaf sheath enclosing the spike was completely discoloured and turned brownish black ; the boot leaf showed chlorotic streaks mostly along the midrib extending to the tip of the leaf and in some cases along the veins on 4th day after inoculation. Similar symptoms were observed in infected crop. The causal organism could be isolated from the discoloured portion of leaf sheath which forms the boot but not from chlorotic streaks on the leaf blades. This type of symptom was restricted only to the boot leaf, the leaves below the boot did not show any such streaking. The black point organism failed to cause any infection on the boots, and the ears emerged normally. The grain formation was also noticed in a few ears but the black point was not produced.

In another experiment the spikes were inoculated in the pre-anthesis and dough stages by the dropper method in which the glumes were gently separated by means of a pair of pointed forceps and two drops of spore suspension were put with a dropper. This ensured the introduction of spores between the glumes and within the florets.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Separate droppers were used for each inocluation. After inoculation the ears were subjected to high humidity for three days by covering them with alkathene bags.

In the case of spikes inoculated in the pre-anthesis stage, *A. triticina* produced heavy infection on the awns including the glumes and there was no formation of the grain, while *A. tenuis* failed to produce any infection on the glumes and the grain formation was normal. Only when the inoculations were made in dough stage did *A. tenuis* produce the typical symptoms of black point disease, the blackening was restricted to the embryo, and the glumes and awns remained green, whereas with *A. triticina* the glumes including the awns as well the grain were severely infected, and the blackening often extended to almost the whole grain. The respective organisms were always reisolated from the seed.

After the grain had been fully formed, but the ear was still green, the glumes and awns were infected by *A. triticina* but not by *A. tenuis*, and neither of the two organisms infected the fully formed grain.

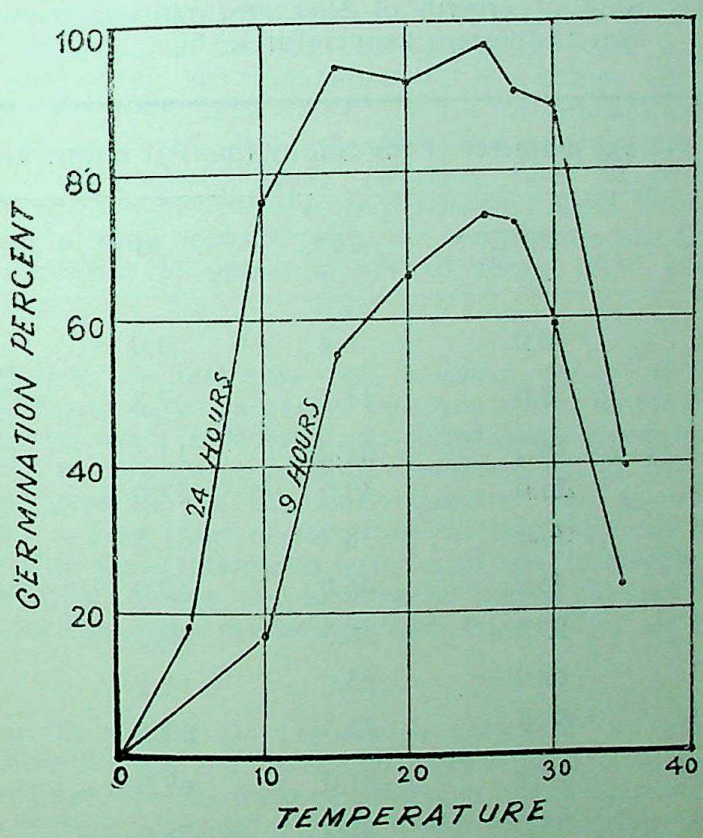


Fig. 2. Relation of temperature to rate of germination of conidia of *Alternaria triticina* after 9 hours and 24 hours

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Germination of conidia : The conidia taken from 10 days' old plate culture grown on standard nutrient agar germinated after 3 hours in water at 24°C. All the cells of the spores produced germ tubes. The spores germinated over a wide range of temperatures (Fig. 2). The optimum was not well defined and more than 90 per cent of the spores germinated between 15°C and 27°C within 24 hours. There was appreciable germination of conidia at 5°C while 76 per cent germinated at 10°C. Within 9 hours, about 75 per cent of the spores germinated between 25° and 27°C. The percentage of germination gradually decreased above 30°C.

Maximum germination of spores (97.4 per cent) took place when relative humidity was 100 per cent. At 90 per cent and below there was no germination.

Effect of temperature on the growth rate of the pathogen in culture : The influence of temperature on linear growth of the fungus was determined on malt extract agar (Malt extract 30 gms, agar 20 gms ; water 1000 cc.), at 10°, 20°, 25°, 30°, and 35°C. The results are presented in Table 3.

TABLE 3 : Rate of growth of *Alternaria triticina* on malt extract agar at different temperatures.

No. of days	Average diameter of the colony (mm) at temperatures of				
	10°C	20°C	25°C	30°C	35°C
1.	0.0	0.0	6.4	0.0	0.0
2.	5.0	8.0	13.1	7.5	4.0
3.	12.5	16.5	21.2	13.5	7.0
4.	19.5	25.0	30.0	22.0	9.5
5.	24.5	34.5	38.7	31.5	13.5
6.	30.0	45.0	46.0	40.0	16.0
7.	37.5	53.5	56.5	49.5	19.5
8.	44.0	62.0	65.0	55.5	24.0
9.	48.0	67.0	73.5	62.5	29.5
10.	52.0	75.5	81.0	69.0	37.0
11.	54.0	83.0	87.5	76.0	44.5
12.	56.5	—	92.0	82.0	48.5

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

It is evident from Table 3 that the fungus grows in culture media over a fairly wide range of temperatures. The optimum growth of the fungus based upon rate of increase in diameter of the colony appears to be 25°C. The decrease in growth was very rapid from 30 to 35°C. Spores of usual shape and size were produced in abundance at the optimal temperature. At 35°C the sporulation was retarded and abnormal aerial mycelium was produced. There was also no spore formation at the lower end of the temperature range.

Effect of temperature on the degree of infection and disease development : In order to determine the effect of temperature on infection and disease development, susceptible varieties Niphad and Kenphad in the fifth leaf stage were inoculated periodically from November, 1961 to April, 1962. The plants, after inoculation, were incubated in moist chamber for 72 hours. The results obtained are presented in Table 4.

It is obvious from Table 4 that temperature is an important factor in infection and incubation period. The incubation period ranged from 4 to 6 days depending upon the prevailing temperature. In inoculation experiments made between November and April, heavy infection was obtained on plants inoculated on 13th November and 2nd December while the plants inoculated on 18th December gave negative results showing thereby that no infection could be obtained when the average temperature was 18°C and below. When the temperature increased to 20°C and above in January (4th—13th) slight infection was noticed on the lower leaves. Again heavy infection could be obtained in the month of February when the average maximum ranged between 22-23°C. Light infection was obtained when the temperature was above 25°C in March and almost no infection at and above 30°C in the month of April.

Experiment to determine the minimum period of incubation in saturated atmosphere for successful infection : One month old plants of Niphad variety were inoculated by spraying the spore suspension with an atomizer and incubated in moist chamber for 6, 12, 24, 48, 72 and 96 hours. The humidity in the moist chamber was approximately 100 per cent and a free film of moisture was always formed on the leaves. The plants after these treatments were transferred to greenhouse benches. The temperature during the experiment ranged between 20 and 25°C. Suitable controls were maintained and the results are recorded in Table 5.

It can be seen from the results presented in Table 5 that the plants removed from the moist chamber after 6, 12 and 24 hours did not get infected whereas those remaining in humid chamber for 48 hours or longer became diseased. Even though a minimum period of 48 hours in a saturated atmosphere was required for successful infection, the maximum infection was observed only in plants kept for 72 hours. In those plants which were kept in moist chamber for 48 hours

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 4 : Effect of temperature on infection and disease development.

Period of inoculation	Temperature °C				Incubation period in days	Number of leaves inoculated	Number of leaves infected	Severity of infection
	Average maximum	Average minimum	Absolute maximum	Absolute minimum				
13.11.61—22.11.61	25.90	19.44	32.22	16.11	4	78	52	+++
2.12.61—11.12.61	21.93	19.05	25.56	11.10	5	82	59	+++
18.12.62—27.12.62	18.78	12.00	22.22	9.44	—	64	0	—
4.1.62—13.1.62	20.96	10.85	22.27	10.60	6	94	28	+
31.1.62—9.2.62	23.40	15.28	26.67	11.10	5	69	31	+++
15.2.62—24.2.62	22.90	16.05	26.67	16.01	5	97	52	+++
16.3.62—25.3.62	27.10	17.78	32.78	14.44	5	92	54	++
3.4.62—12.4.62	33.28	18.36	41.00	15.50	6	88	12	+

— No infection
+ Slight
++ Moderate
+++ Heavy

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

comparatively fewer spots developed on lower leaves on 5th day whereas numerous chlorotic spots indicating heavy infection were observed on 4th day in plants incubated for 72 hours. The leaves became chlorotic and turned yellow when the plants were allowed to remain for 92 hours in moist chamber. These results clearly show that a humid atmosphere for 72 hours is most suitable for heavy infection.

TABLE 5 : The period of incubation required in moist chamber for the infection of wheat with *Alternaria triticina*.

Period of incubation in moist chamber in hours	Number of leaves		Remarks
	Inoculated	Infected	
6	142	0	No trace of infection
12	121	0	—do—
24	154	0	—do—
48	162	59	Moderate infection
72	147	94	Heavy infection
96	140	97	Leaves became chlorotic

Host range : The host range of the fungus was studied by inoculating fully grown plants of 10 species of grasses belonging to 8 genera viz. *Dicanthium annulatum*, *Heteropogon contortus*, *Panicum antidotale*, *P. repens*, *Pennisetum orientale*, *Arundinella nepalensis*, *Bothriochloa* sp., *Cenchrus* sp., *Aegilops* sp., four varieties of barley (N. P. 13, N. P. 21, K 251 and C 138-2) and two varieties of oat, N. P. Hyb. 1 and N. P. Hyb. 3. In addition, since *Alternaria* spp. are known to have a wide host range, plants belonging to several other families such as *Medicago sativa*, *Melilotus alba*, *Pisium sativum* (Leguminosae) ; *Brassica oleracea* var. *capitata*, *B. oleracea* var. *botrytis*, *B. oleracea* var. *caulo-rapa*, *Raphanus sativus*, *Brassica rapa* (Cruciferae); *Lactuca sativa* (Compositae); *Cucumis sativus* (Cucurbitaceae) and *Lycopersicum esculentum* (Solanaceae) were inoculated in the seedling as well as adult stages. The inoculations were repeated several times but none of the plants tested, other than wheat, got infected,

Varietal reaction : Field observations have revealed that different wheats differ in their susceptibility to *A. triticina*. In order to find out the sources of resistance in available genetic stocks, 30 exotic varieties belonging to 12 species of *Triticum* and 38 Indian wheats which have been released for general cultivation in different parts of the country were tested in the glasshouse with Delhi isolate.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Inoculations were made under as uniform conditions as possible on two month old plants. A susceptible Niphad variety, inoculated simultaneously, as well as uninoculated, served as a control. The results recorded after 10 days of inoculation on 10 to 14 plants of each variety grown in 10″ pots are presented below.

Exotic varieties :

Resistant, Hope (E. 41), H-44 (E. 48), Ceres (E. 50), Thatcher (E. 124), Gabo (E. 569), Cometa Klein (E. 671), Frondoso (E. 771), La Prevision (E. 928), Klein 157 (E. 937), Timstein × Mida (E. 1912), New Federation (E. 63) (*Triticum aestivum*) ; E. 2025, E 2143, E. 470 (*T. durum*) ; American Club (E. 13) (*T. compactum*) ; Khapli (E. 56) (*T. dicoccum*) ; Persian Black (E. 18) (*T. persicum*) ; Polish (E. 17) (*T. polonecum*) ; E. 14 (*T. sphaerococcum*) ; E. 15 (*T. spelta*) ; Cambridge Rivet (E. 20) (*T. turgidum*) ; E. 80 (*T. timopheevi*) ; E. 78 (*T. vavilonianum*), E. 25 *T. monococcum*).

Susceptible : Kenya 184 P. Z. A. I. F. (E. 581), Kenya C. 10854 (E. 220) , Kenya 338 (E. 1951), Gaza (E. 931), E. 4717, E. 4849, E. 5550, E. 5673.

Indian varieties :

Resistant : N. P. 823, N. 824, N. P. 809, N. P. 200 N. P. 4, N. P. 52, Agra local.

Tolerant : N. P. 710, N. P. 718, N. P. 790, N. P. 788, N. P. 761.

Susceptible : N. P. 827, N. P. 828, N. P. 829, N. P. 830, N. P. 400, N. P. 401, N. P. 402, N. P. 404, N. P. 405, N. P. 406, N. P. 407, N. P. 408, N. P. 409, C. 281, C. 591, B. R. 319, Hy-65, RS 31-1, K. 13, Kenphad 21, Kenphad 32, Kathia, Bansi, Gulab, Jaya, Vijaya.

It is apparent from the results presented above that there is abundant breeding material available for the development of wheat varieties resistant to *A. triticina*. Some of the highly rust resistant strains such as Hope (E. 41), H-44 (E. 48), Ceres (E. 50), Thatcher (E. 124), Gabo (E. 569), Cometa Klein (E. 671), Frondoso (E. 771), La Prevision (E. 928) were also found to be resistant to leaf blight. There were both resistant as well as susceptible varieties within the same species. Thus no generalization could be made that a particular species or a group of species is resistant or susceptible. The Indian durums, particulaly the varieties in N. P. 400 series, however, were found to be highly susceptible.

Histological observations : Diseased wheat leaves were examined microscopically to study the relationship of *A. triticina* to the host tissue. It has been found that the spores germinate and produce 1 to 4 germ tubes. Penetration was observed through both upper and lower surfaces of the leaf; conidial germ tubes seldom penetrate

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

through stomata and enter the leaf directly through the epidermis. Round structures are formed at the end of the germ tubes and these function as appressoria. The mycelium is both inter-and intracellular. The outer epidermal cells collapse first and this is followed by the collapse of the mosophyll cells slightly in advance of the invading mycelium. The cells beneath the epidermis collapse and get compressed to such an extent that the thickness of the leaf tissue is greatly reduced as compared to that of a healthy leaf. The tissues become discoloured and somewhat brown in appearance. The conducting vessels i. e. the xylem and phloem of the leaf cells are not invaded by the fungus. The visible symptoms of infection appear 4 to 5 days after inoculation in the form of localised chlorotic specks. The time required for sporulation varies with the prevailing temperature and humidity. When accompanied by high humidity, conidiophores emerge out invariably through the stomata singly or in bundles of 2 to 10 (Fig. 3). The conidia are borne on conidiophores mostly singly and rarely in chains of 2 to 4. The young condia are obclavate and gradually tapering and have very few longitudinal septa.

Perpetuations of the fungus :

Viability under laboratory conditions : Diseased specimens were collected in March 1960 and preserved between blotting papers in the laboratory. When isolations were made from this old dried material in December 1961 all isolates yielded *A. triticina*, the pathogenicity of which was experimentally proved on wheat. This shows that the fungus can remain viable for more than 20 months under laboratory conditions.

Transmission through soil : Since *Alternaria* spp. sporulate abundantly on decaying vegetable matter, soil could be an important harbour of the pathogen. The fungus was grown in soil maize meal medium and incubated for 10 days at 25°C. Six 10″ pots, washed and disinfected with lysol, were filled with sterilized soil. The soil medium containing the inoculum was mixed with sterilized soil in the pots. Ten to fourteen surface sterilized seeds of the susceptible wheat variety Niphad were sown and the plants were allowed to grow till the adult stage in the green house. In the controls the sterilized soil was not inoculated.

Plants remained healthy till they attained the 5th leaf stage but subsequently the lowermost leaves which came in contact with the soil showed symptoms of infection. This indicates that the pathogen if carried to the field may multiply in the soil and subsequently attack the plants when they reach the susceptible stage. The leaves in contact with the soil may thus get infected or the spores may be splashed by rain to the lowermost leaves from which the disease gradually spreads to upper leaves. It appears from this that the crop grown in the infested soil may be of great importance in initiating fresh infections and subsequent dissemination of the inoculum to other fields.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Transmission through seed : In order to study whether this disease can be transmitted through seed, healthy wheat seeds rolled in spores of *A. triticina* were sown in sterilized soil in six 12″ pots. In another set of six pots, the infested seeds were sown in unsterilized soil and proper controls were maintained in each case.

There was no difference in the percentage of germination of wheat seed in the treatments and the controls. The plants were allowed to grow till the ear stage in the green house but all the plants remained healthy. Further experiments will have to be conducted to come to any definite conclusions regarding the frequency of seed transmission. It is possible that the initial inoculum brought with the seed may be built up in the soil with the passage of time to such an extent that the soil itself would become infested, in which case the picture will be the same as described in the earlier experiments where positive infection was obtained in the lower leaves when in contact with the infested soil.

DISCUSSION : The leaf blight disease of wheat appears from the literature to have been in existence in India since long as it was first reported by Kulkarni as early as 1924. Although a definite causal organism had not been identified the disease has been variously attributed to *Alternaria* spp. or *A. tenuis*. The results herein reported have clearly demonstrated that the disease is caused not by *A. tenuis* but by a new species of *Alternaria* which was named as *A. triticina* in an earlier publication by the authors.

Considerable difficulty was experienced in early stages of these studies in obtaining the pure culture of the pathogenic organism. Whenever isolations were made from diseased leaves *A. tenuis* was invariably obtained. As is well known, *A. tenuis* is a heavily-sporulating and fast growing fungus and so it masked the pathogenic organism which is scantily sporulating This may perhaps be one of the reasons for the erroneous conclusion drawn in the earlier investigations in regard to the species of *Alternaria* involved in the causation of the disease. Repeated failures of inoculations with *A. tenuis* reported in this paper have convincingly shown that it is non-pathogenic and comes into culture on account of its occurrence as a saprophyte on the infected leaves. Isolations made from the typical initial infection spots invariably yielded only *A. triticina* which proved to be a virulent pathogen causing the leaf blight disease. The pathogenicity of *A. tenuis* on one week old seedlings reported by Mathur is difficult to explain since in our experiments neither *A. tenuis* nor *A. triticina* could infect the seedlings of that age.

The vigorously growing seedlings showed resistance to *A. triticina* and they could not be infected even by the inoculation method which Neergaard considered to be most sensitive, so much so, that even a faint degree of parasitism would be exhibited. The plants could be infected only after they had reached the third leaf stage and it was observed that always the lower leaf was more vulnerable to infection

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

than the one above it. The flag leaf could not be infected till the ear was formed. It appears that the resistance is directly related to the vigour of the leaf and consequently the susceptibility increases with increase in age. A similar type of seedling resistance to *A. longipes* is known in the case of tobacco. According to Grümmer (1955) decreased tolerance of the pathogen may occur when carbohydrates are depleted because proteins are then hydrolized and the machinery of the cell is weakened. Thus, the analysis of some of the factors that might contribute resistance to the seedlings has to be approached from both the morphological as well as physiological points of view. The disease generally gains foothold after the plants have passed their greatest vigour peak and first makes its appearance on one month old plants.

Inoculation experiments made on the ear by spraying the spore suspension of *A. tenuis*, associated with black point disease, and that of *A. triticina* showed that the former is saprophytic and never infects the glumes, whereas the latter is pathogenic and causes severe infection on the ear. There was no grain formation due to the severe attack of *A. triticina* when inoculations were made by injecting the spore suspension into the glumes in the pre-anthesis stage, whereas in the case of ears inoculated with *A. tenuis* the grain formation was normal and the glumes remained green. The black point disease symptoms were reproduced with *A. tenuis* only when the inoculations were made in dough stage. Thus, it appears that after the grain is fully formed the air-borne spores of *A. tenuis* gain entrance into glumes and form a saprophytic growth on the embryo portion of the seed where the moisture is sufficiently high. The grain was also infected with *A. triticina* when inoculated in dough stage, but the infection was of a different kind as compared to that produced by *A. tenuis* where the blackening was restricted to the embryo portion. The infection by *A. triticina* extended to the whole of the grain.

A. triticina produced chlorotic streaks on the flag leaf along the mid rib and the veins extending up to the tip when the inoculum was injected into the boot. Isolations made from such chlorotic streaks did not reveal any fungus whereas the organism could be obtained from the discoloured portions of the boot. It appears that some substance is produced by the infection of *A. triticina* and translocated upwards along the flag leaf, ahead of the fungus growth, and causes the streaks. Similar symptom was also observed on the flag leaf under field conditions when the crop was severely infected.

The disease first makes its appearance in the month of November when the plants are 6 to 7 weeks old and assumes severe form in February when they are sufficiently mature.

The degree of infection depends upon weather conditions, and the age and vigour of the plant. Under favourable conditons the leaf spots increase in size and number, and leaves beginning with the lower ones gradually die until only a few spotted leaves remain at the top. It has been shown that the maximum disease development takes place

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

at temperatures ranging between 20-25°C. There is a close correlation between temperature at which the maximum infection develops and the temperature prevailing in the month of February. The optimum temperature for infection was somewhat close to the optimum growth of the fungus in culture. The minimum temperature for the infection was shown to be in the region of 19°C. The disease is therefore held in check in December and January due to the low temperatures prevailing during these months. The minimum temperature required for infection was somewhat higher than the minimum temperature for the growth of the fungus in culture. It is likely that the attack occurs as the temperature increases to 20°C. The maximum infection was obtained when the plants were incubated in moist chamber for 72 hours at 100 per cent relative humidity, although even 48 hours moisture was sufficient to cause infection. The disease takes explosive turn after a shower of rain. The severity of the disease in the month of February could thus be attributed to the favourable temperature and humidity as well as to the maturity of the crop.

Observations in the field and infection experiments in glasshouse indicated that wheat is the only susceptible host for *A. triticina*. It is generally believed that the host range of many parasitic *Alternaria* spp. is rather wide, but Dr. Neergaard in a personal communication has stated that there are some species which seem to be more or less specific for the host.

Wheat is grown in winter. There is no wheat during summer but inspite of the absence of any other known host for *A. triticina* the disease recurs every year in the field. The pathogen could perhaps oversummer in the debris that is left lying in the field or seeds sown along with the infected leaf bits may introduce the fungus in the soil where the buildup of inoculum may take place. The spores so produced would infect the lower leaves which are more susceptible than the vigorously growing upper leaves. It has been invariably observed in the field that the lower leaves are the first to be infected. In the present study the experimental evidence that the fungus can cause infection on the lower leaves in contact with the infested soil in the pots is in support of the above view. However, it is necessary to investigate the part played by seed in the perpetuation of the disease so as to arrive at a definite conclusion and devise suitable control measures. Pathogenicity tests on available genetic stock have shown that resistant material is available for utilisation in the breeding programme.

SUMMARY

The pathogenicity of Alternaria isolated from the leaf blight affected leaves and identified earlier as *A. triticina* has been indisputably proved on one month old wheat plants on which the disease first appears as localized leaf spots followed by blightening which is the most pronounced symptom under field conditions. Inoculation experiments with isolates from different parts of the country confirmed that *A. triticina* is a virulent pathogen on wheat. *A. tenuis* which comes

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

into picture as contaminant in the isolations and which was erroneously regarded as the causal organism proved here to be non-pathogenic both on the seedlings and mature plants. Seedlings were found to be resistant to *A. triticina* and the susceptibility of the plants increased with increase in age.

It has been shown experimentally that *A. tenuis* associated with black point of wheat does not cause the leaf blight and is confined to the seed alone. Results of inoculation on the ear with *A. tenuis* associated with black point and *A. triticina* showed that the former comes as a saprophyte after the grain is fully formed and does not infect the glumes or the awns whereas the latter attacks the ears at all stages of grain formation.

Conidia of *A. triticina* germinate at temperature ranging from 5 to 35°C. The optimum was not well defined and varied from 15 to 27°C. Hundred per cent humidity is required for the maximum germination. The optimum temperature for growth in pure culture was found to be 25°C. Maximum development of disease took place around 25°C. Moisture has been found to have profound effect on infection. A minimum period of 48 hours of saturated atmosphere is prerequisite for successful infection.

Host range studies showed that wheat is the only host for *A. triticina*. Wheat varieties differed markedly in their reaction to the pathogen. Some of the rust resistant genetic stocks such as Hope (E.41), H-44 (E. 48), Ceres (E. 50), Thatcher (E. 124), Gabo (E. 569), Cometa Klein (E. 671), Frondoso (E. 771), La Prevision (E. 928) etc. have been proved to be resistant and the varieties Kenphad 21, Niphad 4, Vijaya, Jaya, N. P. 827, N. P. 828, N. P. 830, C 281, C 591, Gaza (E. 931) were highly susceptible to this disease.

Division of Mycology and Plant Pathology,
Indian Agricultural Research Institute,
New Delhi.

References

ANONYMOUS (1961) *Proceeding of the All—India Wheat Research Workers' Seminar, Delhi.*

BENER RAJ. R. (1960) Alternaria disease of wheat in Andhra Pradesh. *Andhra Agri. J.*, **7** : 114-115.

BOSE, S. R. (1956) Alternaria within the pericarp of the wheat seed. *Nature*, Lond., **178** : 4534 : 640-641.

GRUMMER, G. (1955) Die Bezievhungen zwischen dem Eiweistoffwechsel von Kulturpflanzen und Anfalligkeit gegen parasitische. *Phytopath Z.*, **24** : 1-42.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

KULKARNI, G. S. (1924) Report of work done in the Plant Pathology Section during the year 1922-23. *Ann. Rept. Dept. Agric. Bombay Presidency for the year* 1922-23, 167-171.

MATHUR, R. S. (1956) Alternaria leaf spot of wheat in U. P. *Agric. and Anim. Husb.* U. P., **6** : 12-14.

MEHTA, P. R. (1950) Some new diseases of plants of economic importance in U. P. *Plant Protc. Bull.* New Delhi, **2** : 50-51.

MCLEAN, R. C. AND W. R. I. COOK, (1941) *Plant Science Formulae.* Macmillan and Co. Limited, London, pp. 203.

MCRAE, W. (1924) Economic Botany, Part III. Mycology *Ann. Rept. Board Scientific Advice*, India, 1922-23, 31-35.

————(1933) Report of the Imperial Mycologist *Scient. Reports. Imper. Inst. Agric. Res.* Pusa, 1931-32, 122-140.

NEERGAARD, P. (1945) *Danish species of Alternaria and Stemphylium.* Humphruy Millford, Oxford University Press, London.

PRASADA, R. AND A. S. PRABHU (1962) Leaf blight of wheat caused by a new species of *Alternaria*. *Indian Phytopath.*, **15** : 292-293.

PRASADA, R. AND A. S. PRABHU (1964) Leaf blight of wheat. *FAO Plant Protc. Bull.*, **12** : 117.

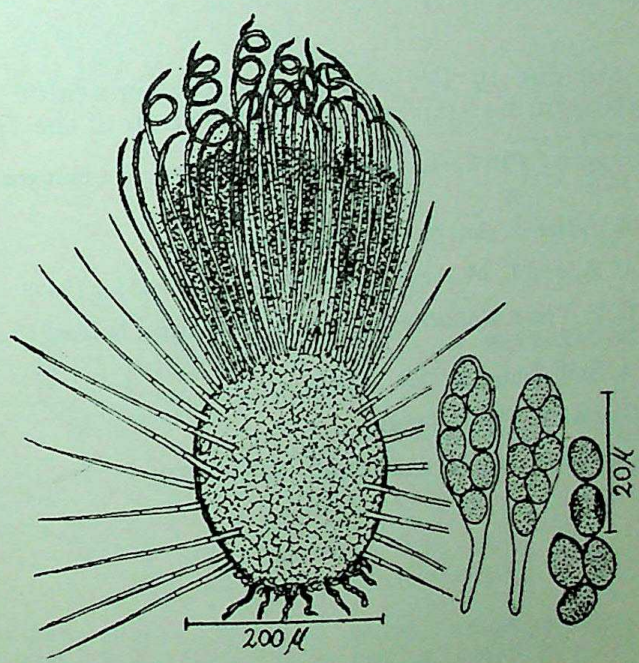
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

Studies on Chaetomium bostrychodes Zopf.—A. K. Roy, Division of Mycology, Department of Agriculture, Assam, Jorhat.—*Chaetomium bostrychodes* Zopf. has been reported on decaying animal body, potato tuber, dung in Germany[5], damaged books in Russia[1] and Palestine[2], butter in Ireland[4], military cotton fabrics in U.S.A.[3] and black cotton soil in Madras[7]. From Borbheta (near Jorhat) Assam, it was isolated from a petridish as a contaminant. Subsequently it was tried to find out the source of inoculum and for that purpose different soil samples, compost heaps, cowdung etc. were collected from the nearby localities and plated by dilution method but all the attempts were fruitless. However, in one plating it was found on decomposed paper near the laboratory.

On P. D. A., perithecia gregarious, brown, composed of single layered pseudoparenchymatous cells, oval, 133-235 μ in height and 124-200 μ in breadth; attach loosely with the substratum by flexuous, light brown, septate, rhizoid like hyphae 2.4-3.5 μ broad. Apical setae brown, septate, coiled at the top like spring, 3.3-5 μ broad; lateral setae straight, stiff, brown, gradually pointed and lighter at the tip, 116-269 $\times$ 3.3-4.5 μ. Asci clavate, stalked, hyaline, thin walled, evanascent, octasporous 34-48 $\times$ 8.3-13 μ ; ascospores greyish brown to brownish grey, ovoid to oval, 5.5-8.0 $\times$ 5-6.5 μ, arranged irregularly biserriate. The culture has been deposited in the National Type Culture Collection, New Delhi, accession No. 1232.



CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Five media, viz.. P. D. A., Oat meal agar, Richards, Czapek's and Brown's agar were tested to see their effects on growth and sporulation. Richard's medium supported the best growth which was almost equal in P. D. A., moderate in Brown's and Oat meal agar and poor in Czapek's medium. Though different gradations have been made but on the whole the rate of growth was slow. Perithecia started forming after 3-4 days in P. D. A. and Oat meal agar and after 7 days in Brown's agar. There was faint zonation in P. D. A. In all the media colonies were water translucent, effuse, almost nil aerial growth and formed a plectenchymatic thin stroma on the surface.

Seven ranges of pH from 4 to 10 were tried in Richard's medium of which 7 supported the best growth and declined on either side. There was almost no growth in 4 and 10. In order of preference 7 was followed by 6, 5, 8, and then 9. Colonies in 6 and 7 were thick and somewhat convulated which were thin in 8. In other cases growth was very sparce.

Germination of ascospores was attempted in 2 p. c. glucose and sucrose, 0.01, 0.001, 0.0001 p. c. of gibberellic acid and in distilled water following the method of Sarkar[6]. Percentage of germination was 88 in sucrose, 83 in glucose as against 66 in distilled water after 24 hours. There was no germination in any concentration of gibberellic acid. The spores germinated by a single polar germ tube which was somewhat swollen at the point of origin and took a slight curve and then straightened up mostly along the opposite direction or sometimes same direction. Size of germ tubes were 75-192 $\times$ 2 μ in sucrose, 112-188 $\times$ 2 μ in glucose and 46-50 $\times$ 1.6 μ in distilled water.

Thanks are due to Dr. Booth of Commonwealth Mycological Institute, London, for his kind help in identification of the fungus.

[1]Bakhtin. V. S. Proc. Pan Soviet Congress of Botanists in Leningrad in January, 1928 : 169-170 (1928).

[2]Goldzwing, M. *Palest. J. Bot. Ser.* **4** : 107-108. (1947).

[3]Greathouse, G. A. and L. M. Ames. *Mycologia* **37** : 138-155. (1945).

[4]Grimes, M., C. E. Violet Kennelley and H. A. Cummins. Scient. *Proc. Roy. Dublin Soc.*, N. S. **19** : 549-569. (1930)

[5]Saccardo, P. A. Syll. Fung., **1** : 224. (1882).

[6]Sarkar, A. *Lloydia* **23** : 97-101. (1960).

[7]Subramanian, C. V. *J. Madras Univ.* B, **22** : 209. (1952).

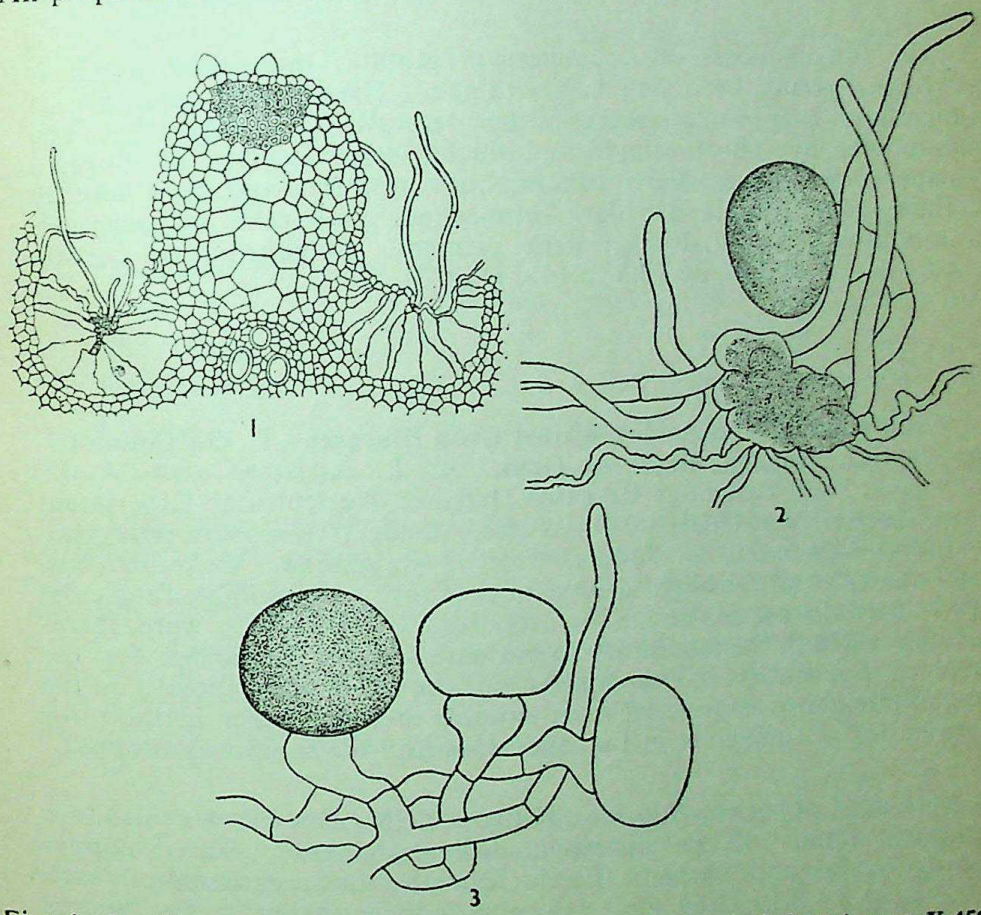
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Host Pathogen Relationship in Nigrospora Disease of Rice—ASOK KUMAR KAR, Department of Botany. Presidency College, Calcutta-12.—During the survey of diseases of rice (*Oryza sativa L.*) in the Sundarbans in December, 1963 dark streaks and spots with powdery appearance were observed on the surface of leaf blades, sheathing leaf petioles, and on glumes of grains of some otherwise disease-free plants. A similar diseased condition was also noticed on young leaves which remained partly enclosed within the sheathing petioles, and on immature grains of rice. These young leaves remained rolled and did not sufficiently grow to become exposed to the external conditions.

This disease is caused by the fungus *Nigrospora oryzae* (Berk. & Br.) Petch, and has been widely reported from the rice growing countries of the world. The primary object of the present investigation was to study this rice disease with special reference to the host pathogen relationship.

80 freshly collected naturally infected samples were studied. No artificial infection was carried out. Mounts were made both by taking scraping from the lesions and by freehand sectioning of diseased material. All preparations were mounted in lactophenol-cotton blue.



Figs. 1—3. *Nigrospora oryzae*. 1. Cross seetion through an infected area. X 450 2. A sporodochium-like body bearing conidiophore and conidium. X 1000 3. Septate mycelium, conidiophores, and conidia, X 1000.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SYMPTOMS : Dark streaks and powdery spots of variable dimensions are visible with the unaided eye on the surface of leaf blades, sheathing leaf petioles, and the glumes of grains. Closer examination under dissecting microscope reveals that the streaks are minute, dark pustules developed in linear series and coalescent pustules form the powdery spots. The pustules are dark in colour and are more prevalent along furrows than the ridges of the host surface. The dark pustules are powdery masses of conidia developed by the mycelium of the pathogen that has already gained entrance in the host tissue. Criticial microscopic examinations of scrapings of pustules indicate the nature of conidia, conidiophores, and mycelium.

BEHAVIOUR OF PATHOGEN WITHIN THE HOST : Sections reveal that the infection is very superficial, specific, and localized. The pathogen enters through stomata, is restricted particularly along the outer layers of the parenchyma with very little tendency to penetrate deeper (Fig. 1). The mycelium ramifies along the parenchymatous tissue and produces sporodochium-like bodies after rupturing the host tissue (Fig. 2). The sclerenchyma adjoining the parenchyma remains unaffected. The pathogen induces mild infection resulting in little damage to the host tissue.

The fungus possesses characteristic septate mycelium which is hyaline when young, becoming dark with age. The ultimate branches of the mycelium bear conidiophores either laterally or terminally. The conidiophores are short, simple, and inflated below the tip ; exhibiting a jar-shaped appearance. Each conidiophore produces apically a solitary conidium. The conidia are dark, one-called, globose to subspherical, and smooth-walled with distinct dark pigment. Dimensions of conidia range from 11.5 to 15 μ by 10.5 to 14 μ (Fig. 3).

———

Evaluation of Fungicides against Crop Diseases—I On Colletotrichum capsici causing blight of Guar.—K. L. KOTHARI AND M. K. BHATNAGAR, Plant Pathology Research Division, Agricultural Experiment station, University of Udaipur, Rajasthan.—Guar (*Cyamopsis tetragonoloba* Taub.) is an important fodder crop of Rajasthan. *Colletotrichum capsici* causes a serious blight disease in the crop. No suitable fungicidal control of the disease has so far been reported. The studies were, therefore, undertaken in the laboratory to evaluate suitable fungicides for the control of the disease. The studies on the effect of fungicides on the spore germination is considered more suitable then any other method for the evaluation of fungicides in the laboratory and the same was adopted.

Fytolan (Copper oxychloride), Dithane Z-78 (Zinc ethylenebisdithio carbamate), Ziram (Zinc dimethyl dithiocarbamate), Kirti copper (Cuprous oxide) and Ferbam (Ferric dimethyl-dithiocarbamate), each were taken at 2,4,8,16,32,64 and 128 ppm concentrations. The spores for study were taken from ten day old culture of *Colletotrichum capsici* on P. D. A.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The method used was a little modification of that described by McCallan[2], and Lilly and Barnett[1]. Flasks containing 100 ml. of 2 per cent agar in distilled water were autoclaved. The solution of fungicide was carefully added to each flask, just after autoclaving, keeping in view the final concentration to be prepared. The medium was plated in three petridishes in each case. A homogenous spore suspension (20-30 spores under low power of microscope, ×10) was prepared in sterile distilled water. After solidification of medium 5 ml. of the spore suspension was poured in the centre of each petridish and was carefully spread out. The covered plates were incubated at 32°C for 24 hours before examination.

The plates were examined under the microscope. Four readings were taken from each plate. The average percentage inhibition of spores so observed is presented below in the table.

TABLE : Percentage inhibition of the spore germination of *Colletotrichum capsici* in various concentrations of different fungicides.

Fungicide	Concentrations in p. p. m.						
	2	4	8	16	32	64	128
Fytolan.	12	25	25	76	86	100	100
Dithane Z-78	12	12	14	67	77	90	100
Ziram	10	100	100	100	100	100	100
Kirti copper.	90	93	93	98	100	100	100
Ferbam	100	100	100	100	100	100	100
Control	8	(Average of 12 readings from 3 plates)					

The results indicate that Ferbam is highly effective against the spores of *Colletotrichum capsici* and has completely inhibited the germination even at 2 ppm level. Ziram supported excellent germination at 2 ppm level but has absolutely lysed the spores at 4 ppm concentration. Kirti-copper, on the contrary, did not support good germination even at the lowest level though the complete inhibition was not attained lower than 32 ppm level. Fytolan and Dithane Z-78 have shown total inhibition at 64 and 128 ppm levels respectively.

ACKNOWLEDGEMENT : Authors are grateful to Dr. N. Prasad, Dean, College of Agriculture, Jobner for guidance and Dr. B. K. Srivastava, Director Research, University of Udaipur, Udaipur for encouragement.

[1]Lilly V.G and Barnett H. L. (1951). Physiology of Fungi. pp. 245-263. Mc Graw Hill Book Co. INC., London.

[2]McCallan, S. E. A. Bioassy of Agricultural Fungicides *Agr.* Chemical, 1947.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Effect of Trace Elements on Rhizoctonia spp.—L. N. DAFTARI, Regional Research Station, Banswara, Rajasthan.—The role of trace elements on the growth and sporulation of fungi has been recognised since long. Blank Lister (1) reported that Cu, Mn and Fe reduced the myclelial production in case of *Phymatotrichum omnivorum*. Perlman (2) observed that Zn induced the pigment formation in *Sclerotium delphinni*. In the following investigations, effect of certain elements on the growth and sclerotial formation of *Rhizoctonia solani* and *Rhizoctonia bataticola* have been studied and results reported thereof.

Asthana and Hawaker's medium was used throughout the study which consisted of glucose 5 gms ; KNO_3 3.5 gms ; KH_2PO_4 1.75 gms ; and $MgSO_4$ 0.75 gms. in 1,000 c. c. of distilled water. In solid medium 20 gms. of agar agar was added in one litre of medium. The medium was purified by addition of 15 gms. of $CaCO_3$ per litre and antoclaved at 10 lb. pressure for 10 minutes and filtered while hot. 5 ml. of 1. ppm. strength of Cu, Mn, Fe and Zn in the form of sulphates was added to 15 ml. of the medium before sterilization and each 20 ml. of the medium was sterilised in 100 ml. conical flask at 13 lbs pressure for 10 minutes. The pH of the medium was adjusted to 5.4 before sterilization. In case of solid medium 20 ml. of sterilised medium was poured in sterilised petriplates. Before inoculations all media were incubated at room temperature for 36 hrs. The inoculations were made with 3 days old cultures, grown on P. D. A. slants. Equal amount of culture was inoculated in each flask. The experiments were repeated thrice with three sets of plates and flasks. The inoculated flasks and plates were incubated at 30°C. and final observations were recorded.

(a) The role of Cu, Fe, Mn, and Zn on the growth and sclerotial production of *R. solani* and *R. bataticola*.

TABLE 1

Different trace elemets in 1 ppm. conc.	*Rhizoctonia solani*		*Rhizoctonia bataticola*	
	Av. dry wt. in mgs after 17 days	sclerotial formation after 17 days	Av. dry wt in mgs after 17 days	Sclerotial formation after 17 days
Cu	48	0	43	++
Fe	151	++++	56	++++
Mn	125	++++	73	++++
Zn	128	++++	45	++++
Control	113	+++	74	+++

0=No sclerotia + very poor, ++ Poor, +++ medium ; ++++abundant.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(b) Linear growth of *R. solani* and *R. bataticola* at different concentrations of copper in the form of copper sulphate and copper acetate.

TABLE 2

Amount of Cu in ppm. (in the form of $CuSO_4$	Average diameter in m. m. after 4 days.	
	R. solani	*R. bataticola.*
3.9	89	86
19.5	84	79
39	—	68
78	—	40
117	—	—
Amount of Cu in ppm. (in the form of copper acetate.)		
3.5	77	88
17.5	—	43
35	—	—
70	—	—
105	—	—
Control	71	85

From Table No. 1 it is clear that both the fungi showed reduced growth and sclerotial formation in 1 ppm. concentration of copper in the form of copper sulphate. While in case of *R solani* it has been observed that the mycelial formation was accelerated with Fe, Mn and Zn. Results of Table No. 2 show that with increase of Cu concentration in the medium, growth decreased after a limit both with copper sulphate and copper acetate. Copper as copper actetate proved more toxic than copper as copper sulphate. *Rhizoctonia bataticola* was found more tolerant to copper than *Rhizoctonia solani* at higher concentrations.

I express my deep sense of gratitude to late Dr. R. P. Asthana, Principal and Professor of plant pathology, college of Agriculture, Nagpur for guidance during the course of studies, and thanks due to Dr. Sehgal A. P. P., Kota, for going through the manuscript of the paper.

[1]Blank Lister *M. Jour. Agri. Res.* 62 : 129-159, (1941).

[2]Perlman *D. Amer. Jour. Bot.* 35 : 360-63, (1948).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Dry Rot of Maize caused by *Diplodia macrospora* **Earle**—K. S. DHANRAJ, Division of Mycology and Plant Pathology. Indian Agricultural Research Institute, New Delhi.—During a disease survey of Kalimpong and Sikkim region in the month of August 1964, the author observed dry rot of maize cobs caused by *Diplodia macrospora*. The disease was fairly common at Kalimpong.

Badly diseased cobs affected with *D. macrospora* are shrunken and shrivelled and the husks are tightly glued to the kernels by the white mold which spreads through the kernels, causing light coloured dry rot. On opening the outer husks, numerous black pycnidia are found scattered all over the inner husks.[1] By harvest time early infected cobs are completely rotted and are light in weight. The kernels become brownish. The symptoms described appear as early as the latter part of August in Sikkim and Kalimpong.

The pycnidia are scattered, carbonaceous, flask-shaped, ostiolate, erumpent, elevating and rupturing the epidermis and measure 150-450 μ. pores long, dark fuliginous, irregularly clavate or cylindrical clavate, unequally uniseptate but 2- or 3- septate forms also occur, scarcely constricted, each cell often biguttulate, 43-95 x 6-13 μ mostly 60-75 x 8-10μ. In other characters also the fungus agrees with the description given by Earle.[2]

The earlier report of cob rot from India is by Kapoor[3], the casual organism being *D. zeae* (Schw.) Lev., which he has seen on cobs collected from Sikkim. In *D. zeae* the dark brown pycnidia are formed chiefly on the pericarp of the kernels. These pycnidia are irregular to subglobose, having a well-defined beak protruding though the host tissue. Spores are cylindric to ovate, both endes rounded, olive brown, straight or slightly curved and measure, 25-31 × 5-6 μ.

It will be seen that the present organism described differs from *D. zeae* in colour, shape and size of the spores. But it agrees in all respects with *D. macrospora*. Cob rot caused by *D. macrospora* seems to be the first record in India. The specimen has been deposited in the *Herbarium Cryptogamae Indiae Orientalis*.

ACKNOWLEDGEMENT : Grateful thanks are due to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology for his encouragement and helpful criticism.

---

[1]Sprague, R. Diseases of Cereals and Grasses in North America. The Ronald Press Company, New York (1950).

[2]Earle, F. S. *Bull. Torr. Bot. Club.* **24** (1897).

[3]Kapoor, J. N. *Indian Phytopath.* **16** : (1963).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ABSTRACTS OF PAPERS PRESENTED AT THE SYMPOSIA HELD AT CHANDIGARH ON 1ST AND 2ND JANUARY 1966.

I. Diseases of Maize and their control.

Chemical Control of Helminthosporium turcicum Blight of Maize H. S., SOHI S. L. SHARMA AND B. R. VERMA—*Agriculture Department H. P. Solan*—Maize, an important cereal crop of Himachal Pradesh, suffers considerable losses in yield due to *Helminthosporium turcicum* Pass., which becomes destructive in certain localities during certain years. The disease generally appears during the end of July, becomes destructive during August and completely blights the foliage during September. To control the disease replicated trial was laid out during 1962 at Dhaulakuan (550 m. m. s. l.) in Sirmur District and during 1963 and 1964 at Solan(1500 m. m. s. l.) in Mahasu District with six different fungicides including inorganic fungicides of copper such as oxychlorides, oxides at the rate of 3 lbs. per 100 gal. and Bordeaux mixture 4 : 4 : 50 and organic fungicides, viz., Zineb at 2 lbs. per 100 gal. and Captan at 3 lbs. per 100 gal. The fungicides were sprayed on Ganga Hybrid Makka 101 at fortnightly intervals from the first appearance of the disease. In all four sprayings were given. It was observed that all copper sprays were highly toxic to the plants, severely injuring and burning the foliage within a few hours of spraying. Zineb and Captan proved most efficaceous in reducing the disease intensity from moderate to slight during 1962 and 1963 and from light to very slight during 1964. Both the fungicides improved the yield as well, Zineb giving an increase of 2, 11 and 15 per cent and Captan 26, 2 and 19 per cent over the control during the above periods. However, the yield differences among them and the control were not statistically significant.

———

A Phaeosphaeria Leaf Spot of Maize. M. S. RANE, M. M. PAYAK AND B. L. RENFRO—*I. A. R. I., New Delhi*—A leaf spot disease was commonly observed in Sikkim, Kalimpong (West Bengal) and in the Tarai area of Uttar Pradesh, on Amarillo de Cuba, Ganga Hybrid Makka 3 and other genotypes of maize in the 1965 *Kharif* season. The disease is characterized by the production of round, elongate or oblong spots or lesions on the leaves. The lesions have prominent chlorotic or bleached areas surrounded by brownish coloured margins. Perithecia are found concentrated in large numbers in the lesions and occasionally pycnidia also develop. Pycnidia containing conidia of *Phyllosticta* readily developed when single ascospores were cultured.When conidia were used as inoculum, mature leisons agreeing with those described above developed in about 15 days on leaves of Ganga 3. Again pycnidial formation was

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

sparse and formed later. A maize leaf spot disease caused by *Sphaerulina maydis* was reported by Hennings in 1902 from Brazil and the characters of the perithecia, asci and ascospores as found herein agree with those described by Hennings. However, it now appears more correct to place the pathogen in the genus *Phaeosphaeria* and accordingly the new combination *Phaeosphaeria maydis* (P. Hennings) Rane, Payak and Renfo is proposed. Earlier in India the symptoms associated with the Phaeosphaeria disease were mistaken for those caused by *Helminthosporium carbonum* and by *Leptosphaeria* spp. So far as is known, the disease has not been reported on maize from anywhere else in the world. The genus *Phaeosphaeria* was established by Miyake to accomodate the pathogen known to induce "Shiro-hagare-byo" (white disease) of rice in Japan which also has *Phyllostiĉta* (*P. oryzae* Hori.) as the imperfect stage.

Diseases of Maize New to India—M. M. PAYAK AND B. L. RENFRO, *I. A. R. I., New Delhi.*—Eleven diseases of maize (*Zea mays* L.), including five found by A. J. Ullstrup, are recorded and described for the first time in India. Cephalosporium stalk, ear and kernel rot (*C. acremonium* Cda. and possibly other species) was prevalent in Rajasthan, Mysore and Delhi from 1962. An epidemic of charcoal stalk rot [*Macrophomina phaseoli* (Maubl.) Ashby], previously reported only on maize kernels, occurred in Kashmir (1960 *Kharif*) and Hyderabad (1964-65 *Rabi*). A new downy mildew of maize (*Sclerophthora rayssiae* var. Zeae Payak and Renfro has been recorded in nine states. It has been observed since 1962 in varying severities and is considered to be a major disease. A leaf spot disease [*Phaeosphaeria maydis* (P. Henn.) Rane, Payak and Renfro] is widespread in the Eastern and Central Himalayan hills and foothills although it is considered minor in importance. A leaf blotch disease [*Didymella exitialis* (Mol.) Müller] was found near Gangtok, Sikkim in 1965 Kharif in which the symptoms produced and the morphology of the causal organism resemble those previously described from Solan, Himachal Pradesh as incited by *Mycosphaerella* sp. The relative importance and distribution of the disease is not known. A heavy attack of rust occurred in the West Bengal hills and lighter severities were noted in Sikkim during 1965 Kharif. Crowded minute uredia and telia of non-erumpent type were present which agree with those described for *Puccinia polysora* Underwood. The disease poses a threat to maize production and especially so if proved to be caused by *P. polysora*. The diseases found by Ullstrup in India and Sikkim in 1960 were : three leaf sopts caused by *Diplodia macrospora* Earle *Hypochnus sasakii* Shirai and, *Gloeocercospora sorghi* Bain and Edg., ear rot caused by *Rhizoctonia zeae* Voorhees; and perithecia of *Gibberella zeae* (Schw.) Petch on old maize stalks.

Stalk Rot of Maize Caused by Pseudomonas lapsa (Ark) Starr and Burk—S. K. SINHA—*U. P. Agricultural University, Pantnagar*—So far two bacterial species causing stalk rot of the maize have been reported from India. They are *Erwinia dissolvens* (Rosen) Burk. and *Erwinia carotovora* f. sp. *zeae* Sabet. During routine isolations, a third bacterium, *Pseudomonas lapsa* (Ark) Starr and Burk., was isolated at the U. P.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Agricultural University, and its pathogenicity was proved both in the field and in the laboratory. The symptoms developed as a rapid rotting of stalks as well as of leaves resulting in lodged plants. The chief distinguishing characters of this bacterium are : rods measuring 0.5 to 1.5 μ, motile with 1 to 4 polar flagella, gram-negative. Colonies on nutrient agar appear grayish blue, produce a green fluorescent pigment in culture, gelatin liquefied, nitrites produced from nitrates, and acid but no gas produced from glucose, sucrose, maltose, lactose, glycerol, raffinose, mannitol, and salicin.

———

Fungicidal Seed Treatment of Maize in India—C. LAXMINARAYAN, S. K. SINHA, S. S. AUJLA, S. K. CONSUL, N. N SINGH, M. M. PAYAK, Y. L. NENE AND B. L. RENFRO—*Maize Research Station, Hyderabad*—Field tests on the effect of treatment of maize seed were made in 1964 and 1965 with three and five fungicides respectively at four locations. Statistically significant differences in plant stands of treated compared with untreated seed were obtained in *kharif* at Delhi in 1964 but not in 1965, at the Uttar Pradesh Agricultural University (UPAU) for both years, at Bajaura (Kulu valley) Punjab in 1965, and at Hyderabed in 1964 and 1964-65 *Rabi*, but not in 1965. Seed treatment with captan, thiram, Agrosan GN, and ceredon were generally superior. The average stand differences between treated and untreated of the two hybrids were as great as 19, 11, 16 and 14 per cent at the above respective locations for the best fungicidal treatment for a particular trial. A significant difference in grain yield resulted from the increased population only with the thiram treatment at Delhi in 1964 and the thiram and captan treatments at Bajaura in 1965. Significant differences occurred among hybrids for plant stands in five of the eight experiments which was in direct relation with germination test data. Generally, plant emergence was delayed one to two days and flowering by one day in plots seeded with untreated seed. Only 12 per cent of the untreated planted seed had fungi associated (species of *Aspergillus*, *Curvularia*, *Fusarium* and *Penicillium* in 1965 at New Delhi and a *Pythium* sp. and *Fusarium* spp. were associated with poor stands at the UPAU. The fungi associated at Hyderabad in 1965 were *Helminthosporium carbonum* and species of *Fusarium*, *Rhizoctonia*. *Cephalosporium*, *Curvularia* and *Sepedonium*.

———

Helminthosporium rostratum Drechs. On Maize, Sudan Grass and Johnson Grass in India—T. P. BHOWMIK—*I. A. R. I., New Delhi*—Recently Bhowmik and Prasada (1965) reported the occurrence of a new leaf spot of maize due to *Helminthosporium rostratum* Dresch., in Uttar Pradesh. Fully developed lesions on the maize leaves were straw coloured with light brown margins and measured 2-40×2-3 mm. in size. Subsequently, this disease was observed in Andhra Pradesh and Gangtok (Sikkim). Seedlings of Ganga Hybrid Makka I were readily infected by conidial suspensions of isolates of pathogen obtained from these areas. Studies on morphological characters revealed differences in both shape and size of conidia.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In addition, leaf spot of Sudan grass [*Sorghum vulgare* var. *Sudanense* (Piper) Hitche.] and Johnson grass [*S. halepense* (L.) Pers.] was also found to be incited by *H. rostratum*. Leaf samples of Sudan grass were collected from Sirsa (Punjab), Malpura and Durgapura (Rajasthan) while the samples of Johnson grass were obtained from the latter two locations. Foliar symptoms of these hosts were small, elongated light purple coloured lesions of 20 × 3 mm. in size which often contained fructifications. Sorghum isolates were not only pathogenic to their respective hosts but also to seedlings of Ganga 1 (maize). The occurrence of *H. rostratum* on Sudan grass and Johnson grass is newly recorded for India.

———

Influence of Maize Mosaic on Host Development under varying Levels of Potassium Fertility and its Infectivity to Sorghum—S. P. RAYCHAUDHURI, E. K. ROHINI AND M. L. SETH—*I. A. R. I., New Delhi*—Maize plants of the variety Meerut Yellow were inoculated two weeks after seeding in a field experiment at Delhi. Majority of inoculated plants later exhibited mosaic spmptoms and an apparent spread to control plots was observed. The average grain yield was reduced by 32 per cent in virus infected plots as compared to the non-inoculated control.

During *zaid* 1965, field experiments on the effect of varying levels of potassium (0, 15, 30, 45 and 60 lbs./acre) on mosaic infected and healthy plants of hybrid maize variety Ganga 101, showed that potassium has a favourable influence on the vegetative growth of the plant, but none on flowering. Diseased crop showed better response to the addition of potassium than healthy plants. Difference in plant height due to differential levels of potassium became evident 27 to 30 days after seeding whereas the difference in height due to virus infection became statistically significant 42 days after seeding. Infection by maize mosaic virus (MMV) delayed flowering, reduced seed set and reduced subsequent seed germination from 60 per cent (non-inoculated control) to two per cent (MMV treatments).

Nine sorghum varieties were found to be susceptible to maize mosaic. There are indications that several sorghum varieties are resistant to MMV.

———

II. Diseases of Millets and their Control.

Bacterial Diseases of Millets in India—G. RANGASWAMI.—*Agricultural University, Bangalore*. In India, studies on the bacterial diseases of millets were taken up only very recently. Of the important major millets grown in India, sorghum is affected by leaf blotch caused by *Xanthomonas rubrisorghi* Rang. *et al.*, and leaf stripe by *Pseudomonas sorghicola* Rang. *et al.* Bajra or *Pennisetum typhoides* is affected by leaf blotch caused by *X. annamalaiensis* Rang. *et al.*, ragi or *Eleusine coracana* by leaf spot due to *X. eleusinae* Rang. *et al.*, and *Setaria italica* by *X. indica* Rang. *et al.* Besides these diseases, the bacterial pathogens known to occur on *Zea mays* and *Saccharum officinarum* have also been reported to infect some of the millets, either in nature or by artificial inoculation. The need for

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

investigating in detail these and other bacterial diseases of millets is discussed as also the possibilities of controlling the *Xanthomonas* infections with antibiotic sprays are indicated.

———

Studies on Green ear Disease of Bajra (Pennisetum typhoides Stapf. and Hubb) caused by Sclerospora graminicola (Sacc.) Schroet.—M. M. TIWARI AND H. C. ARYA.—*Department of Botany, University of Rajasthan, Jaipur.* Recurrence of the disease was studied by taking seeds from ear-heads which showed different degrees of floral proliferation. Such ear-heads (seeds) were grouped into 4 categories : A = ear-heads from disease free clump ; B = healthy ear-head from a diseased clump ; C = partially proliferated ear head ; and D = totally proliferated ear-head with scattered seeds in between. Field and pot experiments were conducted during *Kharif* season. In pots steam sterilized soil (30 lbs. pressure for 1 hr.) was used. For field experiments the soil was virgin for bajra cultivation. The results obtained showed that high percentage of proliferated ear-heads was obtained in plants raised from D-type of seeds. The results were identical both in the field and pot experiments. An important observation was the appearance of conidial infection which invariably preceded the subsequent green-ear formation. The possibility about the role of conidia in infection was also not ruled out as few of the plants from seeds of healthy ear-heads also showed proliferated ear-heads. Further studies about the precise role of seeds and conidia in the epidemiology of the disease are in progress.

In vitro sudies for the growth of *Sclerospora graminicola* on the established callus tissue of disease free bajra stem showed that the fungus parasitizes the tissue, it grows inter, and intra-cellularly. Proliferated individual flower was also cultured, it grew to form callus tissue and the fungus showed its conidial fructifications.

———

Effect of seed treatments on the viability of jowar (Sorghum vulgare Pers.) and ragi (Eleusine coracana Gaertn.) seeds in storage.—K. A. LUCY CHANNAMMA AND M. H. DELVI.—*Dept. of Agriculture Mysore State Bangalore* The effect of seed treatments on the germination of seeds during storage in respect of externally seed borne diseases viz, *Sphacelotheca sorghi* (Link) Clinton and *Helminthosporium nodulosum* Berk. and Curt. of Jowar and Ragi respectively was studied. Aresan, Tillex dry and Ceresan dry at 0.2 per cent by weight were found almost uniformly effective in case of Jowar whereas in case of Ragi, Agrosan G. N. at 0.2 per cent by weight efficiently preserved the viability.

———

Studies on Zonate leaf spot disease of jowar in India.—S. B. PURANIK AND D. SURYANARAYANA. *Indian Agricultural Research Institute, New Delhi* Detailed investigations on the cultural characters, temperature requirements, effect of ultraviolet irradiations, mode of infection and perpetuation of the Indian isolate of *Gloeocercospora sorghi* were carried out. Ultraviolet irradiations for 15 and 30 seconds inhibited the growth of the sorghum isolate of *G. sorghi* temporarily.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Perpetuation studies indicated that seeds infested (i. e., superficially coated) with spores of the fungus did not give rise to diseased seedlings. Infected debris of the previous crop remaining in the field was able to cause disease.

A score card based on the percentage of leaf area destroyed was developed and utilised for grading 141 sorghum hybrids or varieties against natural infection of zonate leaf spot. Out of these only I. S. 3665 was found to be uninfected.

Fifteen sorghum varieties were tested by artificial inoculation test for their relative susceptibility to the zonate leaf spot fungus. Most of the varieties were found susceptible to various degrees. Only I. S. 3665 seemed to be immune.

———

Varietal reaction of Ragi to Helminthosporium nodulosum Berk. and Curt. A. P. MISRA AND A. BOSE. *Agricultural College, Sabour, Bihar.* The varietal reaction of ragi (*Eleusine coracana*) to *Helminthosporium nodulosum*, isolated at Sabour, was investigated. Seed and soil inoculation as well as infection of one month old seedlings were carried out in pot experiments, using the varieties 54-1, A 402, 61-588, A 407, Local, A 405, Co 1, A 403, A 404 and A 406. The percentage of both seedling blight and leaf spot infection in seedlings was maximum in variety A 406 and minimum in A 405. Under soil inoculation test the growth of plants was markedly stunted. The inhibition of shoot and root lengths was also maximum in variety A 406, which was thus the most susceptible amongst the 10 varieties tested. The varieties 61-588 and A 402 were second and third in regard to their susceptibility to seedling blight and the extent of shoot inhibition. Except for the variety A 406 which was most susceptible to seedling blight and leaf spot infection and also most stunted in shoot and root growth, there was variation in respect of all the four effects in different varieties.

———

The Reaction of the Indian Collection of Eleusine coracana to Pellicularia rolfsii nematode disease complex.—H. C., GOVINDU, B. L. RENFRO, L. A. DIXIT AND L. N. SKOLD. *Agricultural University Bangalore* A total of 793 Indian varieties or strains of *Eleusine coracana* (L.) Gaertn. were seeded in February 1965 at Bangalore, at two nitrogen fertility levels. An epidemic of root rot or wilt, caused by *Pellicularia rolfsii* and parasitic nematodes developed and differences in host reaction were evident six to eight weeks after seeding. Many susceptible plants died within 12 to 15 weeks and highly susceptible plants failed to head. *P. rolfsii* was isolated from both diseased tissue and sclerotia formed on diseased plants, while nematodes of the genera *Trichodorus*, *Pratylenchus*, *Helicotylenchus* and *Aphelenchoides* were isolated from soil taken from the rhizosphere. The *E. coracana* entries were finally classified as 322 resistant, 239 intermediate and 232 susceptible. Good agreement for disease index was obtained between the two fertility levels indicating a uniform distribution of the pathogens and the reliability of data collected. Gross differences in disease reaction

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

existed among varieties originating from different states. Collectively, the per cent resistant entries, in parentheses, for 16 states and Sikkim were: Madras (96), Andhra Pradesh (84), Mysore (72), Orissa (51), Kerala (33), Bihar (16), Madhya Pradesh and Uttar Pradesh (14), Punjab (13), Gujarat (11), Maharashtra (7), Assam-NEFA (6), West Bengal (2) and no resistant varieties or plants from Himachal Pradesh, Jammu and Kashmir, Rajasthan and Sikkim.

———

Reaction of the world collection of sorghum to five important diseases.—N. V. SUNDARAM, B. L. RENFRO, J. P. SINGH AND K. O. RACHIE.—*PIRRCOM centre Coimbatore*—The world collection of sorghum (*Sorghum vulgare* Pers.) was grown during 1961 *Kharif* at Coimbatore (2017 entries), 1963-64 and 1964-65 *Rabi* at Hyderabad (4839 entries) and a modified collection during 1964-65 *Rabi* at Coimbatore (921 entries). Data were obtained on the disease reaction of the full collection to leaf blight [*Trichometaspheria turcica* (Pass.) Luttrell], downy mildew [*Sclerospora sorghi* (Kulk.) Weston and Uppal] and rust (*Puccinia purpurea* Cooke) and on the modified collection to grey leaf spot (*Cercospora sorghi* Ellis and Everhart) and zonate leaf spot (*Gloeocercospora sorghi* Bain and Edgerton). Although immunity was not found, partial resistance was recorded in all groups that had any sizeable representation and 44 entries were rated resistant to all five diseases. Groupwise, a summary of data indicates that entries within *Roxburghii* are moderately resistant to leaf blight, rust and zonate leaf spot; in *Conspicuum* to all five diseases; in *Guineense* to leaf blight and downy mildew; in *Membranaceum* to leaf blight and rust; in *Caffrorum* to downy mildew; in *Nigricans* and *Caudatum* to leaf blight, downy mildew, grey leaf spot and zonate leaf spot; in *Durra* and *Cernuum* to downy mildew; in *Subglabrescens* to downy mildew and rust; and, in *Sudanense* to zonate leaf spot. As a group, *Roxburghii* was particularly susceptible to downy mildew, *Caffrorum* to rust and *Durra* and *Cernuum* to leaf blight and rust.

———

An aspect of microbial control of bajra rust.—S. SINHA AND R. G. KAPOORIA—(*Botany Department, Agra, College, Agra*).—The leaf surface of bajra (*Pennisetum typhoides* Stapf and Hubb.) was found inhabited with spores of several fungi evidently falling on the leaf surfaces from the atmosphere. Some of these fungi were found to inhibit the germination of uredospores of *Puccinia penniseti* Zimm inciting the rust disease of *bajra*. When a mixed suspension of uredospores and the phyllosphere fungi was examined after 24 hours, inhibition of uredospore germination was observed. *Aspergillus japonicus* Saito, *Fusarium oxysporum* Schlect ex Fr., *Chaetomium globosum* Kunze inhibited about 90 per cent or more. *Tolyposporium penicillarieae* caused 63 per cent inhibition of germination while most others were only mildly effective or not effective at all. The antagonistic effect of these fungi was followed up in rust development on host seedlings in glass house experiments. When the seedlings were sprayed with mixtures of these fungi and uredospores of *Puccinia penniseti*, the rust development on the host was

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

considerably inhibited. Approximately 40 per cent control of the disease was observed with *Trichoderma koningi*, *Aspergillus japonicus* and *Aspergillus niger* and 70-80 per cent with *Chaetomium globosum* and *Fusarium oxysporum* when the ratio of the uredospores and the phyllosphere fungus spores was 1 : 1. In a ratio of 2 : 1 of phyllosphere fungi and uredospores, 75 per cent inhibition on disease development was obtained with *Aspergillus japonicus*, *A. niger* and *Fusarium oxysporum* and 100 per cent with *Chaetomium globosum*. However, when the spore ratio is reversed the influence of the leaf fungi is diminished to some extent. This aspect of the rust control deserves further investigation in formulating methods of biological control or in discovering the chemical basis of microbial antagonism.

———

Glasshouse experiments on the chemical control of bajra rust. R. G. KAPOORIA AND S. SINHA.—*Botany Department, Agra College, Agra.* During a laboratory assay of certain chemicals dithane S31, dithane M22, cupramar, ceresan, ziram, acid indolyl-3-butyricum, and O-phenanthroline were found to inhibit uredospore germination of *Puccinia penniseti* Zimm. These chemicals were further tested for their efficacy in rust development on host seedlings in glasshouse experiments. 20 ml. of a 100 ppm solution of each of the above chemicals was sprayed on *Pennisetum typhoides* seedlings at 2-3 leaf stage. The sprays were appliedat the time of inoculation or 24 hours before or afterwards. In pre-inoculation spray schedules, 0-phenanthroline proved phytotoxic at 100 ppm and, therefore, in subsequent sprays, only 10 ppm of this chemical was employed. The plants were inoculated with a mixture of uredospores and talc in the ratio of 1 : 500 by weight through a baby cyclone. The mixture was released in the closed atmosphere of the inoculation chamber above the plants. This facilitated uniform settling of uredospores. Fifteen replications were maintained in each treatment. The disease intensity was recorded on the basis of mean number of pustules in treated and untreated checks 15 days after inoculation. The results showed that all the chemicals, except acid indolyl-3-butyricum, were effective when they were applied to the plants before or at the time of inoculation. However, post-inoculation sprays either were not effective, except ceresan which reduced the disease in tensity to about 55 per cent. This indicates that these chemicals can be used as protectants against the rust development.

———

III. Diseases of Paddy and their Control.

The present position of Physiologic races of Piricularia oryzae Cav in India—N. K. CHAKRABARTI, S. C. MATHUR, J. VEERARAGHAVAN AND S. Y. PADMANABHAN. C. R. R. I., Cuttack.—A study has been in progress at the Central Rice Research Institute, Cuttack since 1961 to determine whether *Piricularia oryzae* Cav., the pathogen causing blast disease of rice, occurs as specia- lized races in India and if so, to determine the number and distribution of such races in the country. In this study so far thirty-six isolates of *P. oryzae* obtained from blast infected rice samples from the principal rice growing States of India, have been tested against fifteen varieties consisting of the set of ten differentials developed in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

U. S. A and five from India. In these tests, thirty distinct physiologic races of the pathogen have been identified. Of the thirty races identified, races 11 and 12 correspond to U. S. races 8 and 16 respectively, on the basis of their reaction on U. S. differentials, while IR 13, 14, 15 and 16 may be termed as biotypes of U. S. race 25 as differentiated further by the use of Indian differentials. The thirty-six isolates of *P. oryzae* were also tested against Japanese set of fourteen differentials with a view to test their suitability for identifying Indian races as well as to evolve an international set of differentials. By using the Japanese differentials, Yakeiko, Aichi Asahi, Ginga and Chokotou, five biotypes of the Indian race 11 could be distinguished. None of the races identified on Japanese differentials corresponds to the races occurring in Japan, but IR. 11C from Maharashtra was somewhat similar in pathogenicity to Race C_2 reported from Japan. A few differentials viz., Te-tep, Tadukan and Zenith were resistant to all the races occurring in India.

———

Control of leaf blight of rice, caused by Xanthomonas oryzae (Uyeda and Ishiyama) Dowson—S. S. JAIN, P. RANGA REDDY AND S. Y. PADMANABHAN.—*C. R. R. I., Cuttack*—Field experiments on the control of bacterial blight have been in progress at the Central Rice Research Institute during 1964 and 1965. In the experiment conducted in 1964 with the rice variety *CRT*. 141, the data (the incidence of the disease was assessed at the 'boot' leaf stage) showed that infection was reduced from 54.2 per cent in the control to 17.5, with *Cu* spraying. But *Cu* had a definite toxic effect on the foliage of this variety. In the *rabbing* treatment the infection was as high as 78.2 per cent, but there were no significant differences in yield amongst the treatments in spite of the wide differences in infection. In 1965, two field experiments were carried out one with *CRT*. 141, and the second with Taichung Native No. 1 (imported from Taiwan) both susceptible to bacterial blight. It was found that dipping of seeds for eight hours in 0.1 per cent Ceresan wet+Streptocycline (antibiotic) at 0.3 gm. in 2.5 gallons of water had a significant effect in controlling the initial infection and that the spread of secondary infection could be checked to a large extent by spraying with antibiotic (3 gm/25 gal.) in *CRT*. 141, while in the case of Taichung Native No. 1 the secondary spread of the disease was checked to some limited extent with antibiotic spraying. From the data on spread of infection taken on three different dates it could be inferred that more frequent sprays at the initial period of infection might be more effective in checking the rapid secondary spread in Taichung Native No. 1. As regards yield, the differences amongst the various treatments were not significant.

———

Uniform Blast Nursery Trials—K. V. S. R. KAMESWAR ROW AND S. Y. PADMANABHAN.—*C.R.R. I. Cuttack*—Testing for blast resistance is currently being carried out in eighteen countries of the world on a uniform basis, based upon the recommendations of an International Committee constituted by the F. A. O. in 1959. In 1963 the method of recording observa-

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

tions was simplified at the International Blast Symposium. The Uniform Blast Nursery technique consists, in essence, of growing a bombardment row of susceptible variety alternating with test varieties in dry seed beds. The seed bed should be two meters wide. Each variety is sown to a length of one meter in the centre leaving half a meter on either side which is sown with a susceptible variety Co-13 in our tests. Approximately 100 grains of each variety are sown in two rows 10 cm apart alternated with 2 rows 5 cm apart of the susceptible variety. Before sowing, the beds are manured at the rate of 40 gm of ammonium sulphate and 40 gm of superphosphate per square meter. It is considered that the Uniform Blast Nursery technique may also be used to eliminate the susceptible population in the hybridization projects. Based upon the above Uniform Blast Nursery technique more than 250 varieties have been tested during the last three years in India in the following seven centres (1) Cuttack (2) Deras (Orissa). (3) Coimbatore (Madras), (4) Kalimpong (West Bengal) (5) Lonavala (Maharashtra), (6) Srinagar (Jammu and Kashmir), and (7) Sirsi (Mysore). Such of those varieties which are resistant in field tests will also be subjected to artificial infection to confirm their field resistance. The resistant varieties could be used as donor parents in future hybridization programmes or for direct introductions in suitable areas.

———

Auxin production by the rice leaf blight fungus, Helminthosporium oryzae and other species of Helminthosporium—A. MAHADEVAN, *Annamalai University, Annamalainagar, Madras State* Auxin synthesizing potential of seven species of *Helminthosporium* viz., *H. oryzae*, *H. gibberos pourm H. sativum*, *H. tetramera*, *H. turcicum*, *H. nodulosum* and *H. setariae*, was tested by growing them in Tryptophan-Czapek's medium for 20 days in dark, extracting the auxin and estimating by spectrophotometric and chromatographic methods. All the species produced IAA in the medium, but there was no IAA secretion in the plain Czapek's medium indicating that tryptophan was the auxin precursor. *H. oryzae* produced proteolytic enzyme in a casein-Czapek's medium and with higher proteolytic activity more auxin resulted. Accumulation of IAA in casein medium is attributed in part to its release from IAA-protein complex by the fungal proteolytic enzymes and to the conversion of tryptophan to IAA. Rice plants infected with *H. oryzae* exhibited higher auxin levels. The possible accumulation of IAA due to breakdown of cell wall proteins by proteolytic enzymes during pathogenesis is suggested.

———

Symptoms, Cause and Control of Khaira Disease of Paddy—Y. L. NENE,—*U. P. Agricultural University, Pantnagar*—A disease of paddy known as *Khaira* occurs commonly in the *tarai* region of Uttar Pradesh. Diseased plants are generally found in low-lying areas. They are stunted, show reddish brown leaf discolouration, and retarded, brown root system about a fortnight after transplanting. To some

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

extent, natural recovery of diseased plants is observed about six weeks after transplanting. Investigations were carried out to find the cause and control of the disease. All attempts to isolate any organism in pure culture failed. Soil pH in areas with diseased plants was found to be between 7.8—8.2, as against 7.0—7.3 in areas with healthy plants. Decomposition of paddy stubbles left over from the previous season or of wheat stubbles, if wheat taken in rotation, contribute to the change in pH towards alkaline side in low-lying areas. Analyses show reduced availability of zinc in soil around diseased plants and affected plants contain less zinc than healthy. It was found that recovery of diseased plants is possible through addition of superphosphate and fresh, easily decomposable organic matter to the soil. Sprays with zinc sulphate mixed with lime as safener give outstanding recovery. The results obtained so far indicate that reduction in the availability of zinc from the soil to the paddy plants as a result of change in soil pH towards alkaline side brought about by decomposing paddy or wheat stubbles and/or possibly binding action of decomposing stubbles is reponsible for the incidence of *Khaira*.

———

Prospects of control of blast disease by forecasting and direct application of fungicides and antibiotics—S. Y. PADMANABHAN, *C. R. R. I. Cuttack*—An economic spray schedule has been recommended for direct control of blast based upon five or six sprayings once in the seedling stage, twice or thrice in the tillering phase, at an interval of ten days and twice during the emergence of the ears at an interval of 5-7 days. The crop is most susceptible at these three stages and needs protection. Cupric and organo—mercuric fungicides are the most effective. The spray fluid should have a concentration of 0.25 per cent of metallic copper or if mercury is used, 0.27 gram of Hg may be used in 1 gallon (0.06 gram in 1 liter). It is usual to use about 100 gallons of spray fluild per acre but with low volume nozzles used with pressure retaining sprays, the volume of spray fluid may be cut down to 25 gallons with the same concentration of chemicals. This would reduce the cost of spraying to about one fourth and thereby make spraying an economical proposition without any significant reduction in the effectiveness of the spray. Antibiotics have also been found to be as effective as Cu or Hg based fungicides in bringing down blast infection. The need for spraying at any one stage can be determined by forecasting blast outbreak through a study of the prevailing meteorological factors viz., a minimum of 20°-24°C temperature occurring in association with a high relative humidity of 90 per cent or above in the morning hours during the susceptible stage of plant growth.

———

Control of blast disease of rice through application of antibiotics—S. Y. PADMANABHAN AND K. V. S. R. KAMESWAR ROW—*C. R. R. I. Cuttack*—A species of *Streptomyces* belonging to the *Flavovirens* group was isolated by the senior author in 1959 which showed strong antagonisim to *Piricularia oryzae* in petri plate culture. The crude mycelial extract of the actinounycete was found to inhibit spore germination of *P.*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

oryzae at 1 ppm dilution. The *Streptomyces* strain was cultured in glucose-asparagine medium and incubated at 32°C for seven days. The antibiotic was extrxacted with absolute alcohol by macerating the mycelium. A yellow liquid thus obtained was allowed to evoporate at 40°C. The crude product obtained was mixed with known quantity of talc powder and preserved. This antibiotic was tried in field experiments to control blast during 1962, 1963, 1964 and 1965 along with well known antifungal antibiotics viz., Antimycin-A, Blastimycin, Hamycin, Thiolutin, Bla-S, Kasumin and Aureofungin. They were compared with standard mercury. and copper fungicides of known efficacy. Four to five sprayings with antibiotics were given in all ; 2– 3 at the tillering phase and two at the ear emergence stage. Spraying was also done with a normal volume pressure retaining knapsack sprayer at the rate of 510 litres/ha. It was seen that of the antibiotics tried Bla-S, Kasumin and CRRI antibiotic proved to be effective as the chemical fungicides tried in controlling blast (neck infection) and increasing the yield.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Nineteenth Annual Report of the Indian Phytopathological Society for the year 1965

Mr. President, members of the I.P.S., Ladies and Gentlemen,

I am presenting herewith the 19th Annual Report of the Society for the year 1965. Sixty two new members joined the Society, 3 as life-members and 59 as ordinary members. Twenty two members discontinued due to non payment of the subscriptions during the year. It is a matter of great regret that Dr. R. P. Asthana, a distinguished member of the Society expired during the period under report. The total number of the members at the end of the year was 405 and that of subscribers 268. Subscription is due from a few members only.

During the year, volumes XVII, No. 4, 1964 and XVIII, Nos. 1-3, 1965 of Indian Phytopathology have been issued. The financial position of the Society continued to be satisfactory. Besides, a sum of Rs. 7,500/- continues to remain in the form of N. S. certificates. The amount to the credit of the Society in the S. B. Account with M/s. National and Grindlays Bank Ltd. (Lloyds Branch), New Delhi, and cash in hand taken together on 30-11-65 was Rs. 22,538.58 p. Thus the assests of the Society amount to Rs. 30,038.58. In addition, several cheques are in the process of collection.

During the year the receipts amount to Rs. 19,586.63 and expenditure to Rs. 12,898.95, thus showing a balance of Rs. 6,687.68. It may be mentioned that no financial assistance was received by the Society from any source during the year. We have paid the cost of printing Vol. XVII, Nos. 3 and 4, 1964 and also of Vol. XVIII, Nos. 1 and 2, 1965. The printing of the journal has been in the hands of M/s. Kapoor Art Press, New Delhi.

Five Council meetings were held during the year.

I am glad to inform you that, 11 more members in India have expressed their desire to become fellows of the Society. They will be introduced in the morning session on 2nd January, 1966.

During the year under report the following lectures were delivered by eminent scientists.

1. 30.1.1965. Dr. John Paul, Glasgow University. 'Cell culture in cell biology research'.
2. 30.4.1965. Dr. G. A. Zentmyer, University of California, at present, at Waite Agricultural Research Institute, University of Adelaide—'Phytophthora Investigations in the Americas'.
3. 24.7.1965. Dr. Y. Komuro, Chief of Institute for Plant Virus Research, Chiba, Japan—'Some important plant virus diseases in Japan'.
4. 22.10.1965. Dr. H. Y. Mohan Ram, Delhi University, Delhi—'Plant Tissue culture—problems and possibilities'.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INDIAN PHYTOPATHO

Receipts and Payments Account for

RECEIPTS

To	*Balance on 1st January, 1964 :*		
	Cash in hand	1,535.39	
	Balance with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi in Savings Account	18,494.22	
	12 Years National Savings Certificates in Safe Custody with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi	5,000.00	25,029.61
,,	*Admission Fees*		500.00
,,	*Life Membership Fees*		1,053.00
,,	*Fellowship Fees*		200.00
,,	*Subscriptions :*		
	1960	10.00	
	1961	20.00	
	1962	40.00	
	1963	441.18	
	1964	2,051.20	
	1965	120.00	
	1966	23.75	2,706.13
,,	*Reprints*		1,653.83
,,	*Symposium Receipts*		237.90
,,	*Journal Subscription*		12,688.29
,,	*Interest on Savings Account*		441.20
			44,509.96

Sd./- **S. P. Raychaudhuri,**

Secretary-Treasurer

New Delhi :

Dated : December 3, 1965.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

LOGICAL SOCIETY, DELHI

the year ended 31st December, 1964

PAYMENTS

By	*Establishment*		1,200.00
,,	*Postage*		2,011.73
,,	*Printing and Stationery*		17,727.47
,,	*Travelling and Conveyance*		277.45
,,	*Bank Charges*		335.45
,,	*Miscellaneous Expenses*		91.44
,,	*Symposium and Mundkur Memorial Lecture*		382.00
,,	*Annual General Meeting Expenses*		182.90
,,	*Audit Fee*		700.00
,,	*Purchase of Back Volumes of Journal*		90.00
,,	*Balance as on 31st December, 1964 :*		
	Cash in hand	146.00	
	Balance with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi—In Savings Account	16,365.48	
	12 Years National Savings Certificates in Safe Custody with National and Grindlays Bank Ltd., Lloyds Branch, New Delhi	5,000.00	21,511.48
			44,509.96

Compiled from the Books of Accounts
and information supplied

Aiyar & Co.

Chartered Accountants

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

We are grateful to Dr. K. D. Bagchee for kindly accepting our invitation to deliver the Third Mundkur Memorial Lecture. I am also grateful to Dr. M. J. Thirumalachar, Dr. R. Prasada, Dr. B. L. Renfro and Dr. P. R. Mehta, for helping in various ways to finalise the programme of International Symposium to be held in December, 1966. I am thankful to my colleagues, Mr. T. K. Nariani, Dr. M. M. Payak, Dr. V. V. Chenulu, Dr. L. M. Joshi, as well as Mr. Girdhari Lall, for help rendered to me whenever sought for. Prof. K. S. Thind has been good enough to make all arrangements for the current session at Chandigarh in an excellent way and we are most grateful to him for the same. Dr. G. Rangaswami, Dr. B. L. Renfro, and Shri S. Y. Padmanabhan are mainly responsible for arranging the symposia during this session for which the Society is thankful to them. Finally, the Society is thankful to the Indian Science Congress and the Punjab University, for giving the facilities to hold our meetings here at Chandigarh.

List of Fellows (F.P.S.I.) of Indian Phytopathological Society elected in the Annual General Meeting of the I.P.S., held at Chandigarh on January 2, 1966

Dalela, G. G.
Dharam Vir
Ghosh, T.
Maheshwari, K. L.
Reddy, G. S.
Srinivasan, K. V.
Damodaran, S. (Miss.)
Gemawat, P. D.
Hingorani, M. K.
Mehta, P. R.
Sahambi, H. S.

The following resolutions were considered by the General body and passed :

1. Resolved that the membership fee be increased to Rs. 20.00 per year and the rate for subscriptions (Institutions etc.) to Rs. 40.00 per year from January, 1966 in view of increased cost of printing of the Journal.

2. Resolved that the existing rule restricting new life membership to 5 per year be removed and any member may be admitted for life membership by paying Rs. 240.00 either in lump sum or in 12 instalments within a year.

3. Resolved that eminent Plant Pathologists of the country who get elected to the office of Presidentship of the Society but have not put in 10 years' continuous membership may be exempted from this rule and be accepted as eligible for the Fellowship of the Indian Phytopathological Society provided the registration fee of Rs. 20/- is paid.

Sd/- S. P. Raychaudhuri

Dated : January 2, 1966. *Secretary-Treasurer.*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MANUSCRIPTS FOR INDIAN PHYTOPATHOLOGY

Contributors are requested to follow the instructions given here while preparing their manuscripts.

MANUSCRIPT COPY : Manuscripts should be typed preferably on white sheets (size $8\frac{1}{2} \times 11$ inches) on one side of the page only with at least $1\frac{1}{4}$ inch wide margin on the left and $\frac{1}{2}$ inch on each of the remaining sides. All sheets in the copy should be of one size only. Even in cases where only a few lines are to be added, full size sheets alone should be used.

The entire manuscript should be clearly typed double-spaced including title, foot notes, quotations, references and legends. The writing style should be simple and direct. Check up the copy for spellings, punctuations, diacretic marks, pagination, etc. All calculations, tables, figures, names, quotations, citations, etc. should be carefully verified before submission. Statistical data likewise must be approved by some one competent in Statistics.

Since tabular matter is expensive to print, it should be reduced to a bare minimum just necessary for substantiating the results. All tables, when included, should be consecutively numbered, and each table should have a heading stating concisely its contents.

The foot notes, if any, should be given in the same page in which their reference appears.

Instructions, if any, either to the Editor or Printer, should not be incorporated in the actual MS copy. These may be given on a separate unnumbered sheet.

The contents of the MSS should have brief headings and should broadly be given in the sequence thus : Title, (in capitals), name or names of authors, Introduction, Material and Methods, Results, Discussion, Summary, Acknowledgements, if any, Institutional Address (on left side of the page arranged flush with the margin), References and Legends for figures.

To comply with the International Rules of Botanical Nomenclature (article 34) now in force, Latin diagnosis must be supplied with each new taxon proposed. It may be emphasised that this need not be a verbatim or literal translation of the full English description. An abbreviated Latin diagnosis giving essential characters only with a full description in English will be preferred. Unnecessary duplication (such as inclusion of collection data) in both the languages should be avoided.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ILLUSTRATIONS : The available space for text figures in *Indian Phytopathology* is 4 inches in width and 6½ inches in length. These therefore should be 4 × 6½ inches when reduced. As many of the figures as possible should be **grouped** and they should invariably be mounted close together leaving **no space in between** for reproduction as a single block. Mounting should be done on stiff white cardboard. Use rubber solution for pasting the figures on the board, as it is not messy.

Illustrations should be numbered consecutively in the order in which they are to appear in the text. The numerical sequence should be maintained even in cases where both photographs and India Ink drawings are included. Thus there should be only one figure bearing any particular number.

For numbering avoid use of typewritten symbols, letters, etc. Instead it can be accomplished through a lettering device (a stencil, for example) or printed letters of numbers may be used for this purpose.

Because of high cost of half tone reproduction, (used mainly for photographs, photomicrograph, etc.), carefully made line drawings in India Ink will be preferred. One page of half tone illustration is permissible per paper or article or its equivalent.

The photographs and line drawings, graphs, charts etc. should not be mounted or grouped together. The former are reproduced as half tone blocks while for the latter zinc-etching process of reproduction is used.

The drawings, done in jet black undiluted India Ink, should be bold enough for half or more reduction. Fine stippling consisting of small dots or dashes may be avoided as it often disappears in reproduction.

A scale may be added with each drawing or a group of drawings drawn at the same magnification. This is helpful for the printer to reduce the original figures to any desired size.

Original drawings will be preferred over their photostat copies since in the latter during reproduction, the background becomes murky and grey.

All unnecessary background and foreground in photographs should be trimmed. The trimmin should be so done that the margins become rectilinear. Trimming which results in having grotesque or crooked margins should be avoided. Unless the edges are straight, the background has to be routed resulting in increased cost. Each figure must have a legend. The legends should be typed in double space on a separate page or pages. These should not be pasted or attached to the figures. It may be noted that the legends go to the printer while the figures are sent to the engraver.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES : All references should be double-spaced throughout. These should be listed in the alphabetical order of the authors' names after the Institutional address at the end of the paper. These may be cited as foot notes when they are four or fewer in number. In the text the references should be referred by date thus : Chaudhury (1949) or (Chaudhury. 1949). A citation should give the name of the author (or authors), his/her (or their) initials, year of publication within parentheses, and then the full title correctly, followed by the name of journal (to be underlined), number of the volume, a colon and page numbers. The diacretic signs and capitalization either in the title or in the name of the journal should be precisely as in the original.

The names of the journals should be abbreviated as given in the 3rd Edition (1952) of the WORLD LIST OF SCIENTIFIC PERIODICALS. The Biological Council of England has issued : A LIST OF ABBREVIATIONS OF THE TITLES OF BIOLOGICAL JOURNALS. Authors not having access to the WORLD LISTS may consult this before finally typing the references for their papers.

If an article has not been seen in the original, then that fact should be clearly stated. When a paper in the name of two or more authors is to be cited, only the initials of the first author are to come after the surname, thus : Mueller, E. and S. K. Bose and not Mueller, E. and Bose, S. K. While citing two or more references from the same Journals and/or of the same authors avoid using words : *Ibid.*, *Loc. cit.*, *etc.* and give the full citation.

———

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological News

The following lectures were delivered to the members of the Indian Phytopathological Society at Indian Agricultural Research Institute, New Delhi.

(i) Prof. E. C. Stakman of U. S. A. on 'Role of Plant Pathology in compaigns against hunger' on February 28, 1966.

(ii) Mr. K. A. Pirozynski of C. M. I., United Kingdom on 'An outline of classification of ascomycetes' on March 1, 1966.

(iii) Dr. T. Mizukami of Japan on 'Bacterial blight of rice caused by *Xanthomonas oryzae*' on March 8, 1966.

(iv) Dr. F. T. Last of Glass House Crops Research Institute, U. K. on 'Viruses infecting fungi with special reference to mushrooms' on March 29, 1966.

Dr. N. C. Joshi, Assistant Mycologist, Directorate of Plant Protection, New Delhi is now in U. S. A. to undergo training on uses of pesticides at the University of California, Davis, under USAID programme.

Dr. N. K. Chakrabarti, Plant Pathologist, Central Rice Research Institute, Cuttack is now at the University of Minnesota, to study fungal Physiology, on a Post-doctoral fellowship of Rockefeller Foundation.

Shri J. S. Gill, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, left for Wageningen, Netherlands to undergo a 3 month training course in Nematology on a fellowship offered by the Netherlands Agricultural Research training centre, Wageningen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Contd. from 2nd cover)

PAGES

A study of interaction between Cephalosporium sp. and Azotobacter chroococcum—*N. S. Subba-Rao and V. Iswaran* 87

Biochemistry of fusarium wilt resistance in cotton 1. The influence of amino acids on fungal growth—*A. Mahadevan* 92

Pathological and epidemiological studies on leaf blight of wheat caused by Alternaria triticina—*A. S. Prabhu and R. Prasada* 95

Phytopathological Notes

Studies on Chaetomium bostrychodes Zopf.—*A. K. Roy* ... 113

Host pathogen relationship in nigrospora disease of rice—*Asok Kumar Kar* 115

Evaluation of fungicides against crop diseases—I. On Colletotrichum capsici causing blight of guar.—*K. L. Kothari and M. K. Bhatnagar* 116

Effect of trace elements on Rhizoctonia spp.—*L. N. Daftari* ... 118

Dry rot of maize caused by Diplodia macrospora Earle—*K. S. Dhanraj* 120

Abstracts of papers presented at the symposia held at Chandigarh on 1st and 2nd January, 1966 ... 121

Eighteenth Annual Report of the Indian Pytopathological Society for the year 1965 133

Manuscripts for Indian Phytopathology (Instructions) ... 137

Phytopathological News 140

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00
Cost of Foreign Art Paper with Printing										
2 Pages	3.12	4.25	5.35	6.50	9.75	13.00	16.00	19.00	26.00	32.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1966

President	... ...	R. N. TANDON
Immediate Past President		M. J. THIRUMALACHAR
Vice-President	... ...	K. S. BHARGAVA
Secretary-Treasurer	... ...	S. P. RAYCHAUDHURI (1965-67)
Joint Secretary	... ..	M. M. PAYAK (1965-67)

COUNCILLORS

Northern Zone	... ...	P. R. MEHTA
Mid-Eastern Zone	... ...	B. S. BAJAJ
Eastern Zone	... ...	B. GANGULY
Central Zone	... ...	T. SREERAMULU
Western Zone	... ...	M. C. SRINIVASAN
Southern Zone	... ...	K. RAMAKRISHNAN

EDITORIAL BOARD FOR 1965–67

R. PRASADA

Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU

L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 40/- (£ 3-10-0 or $ 9.00), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12, (India).

Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

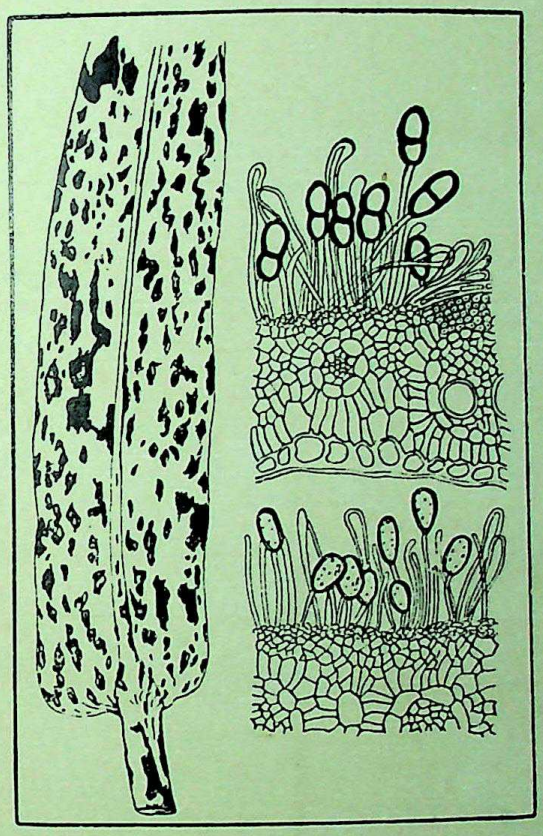
CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

6662

INDIAN PHYTOPATHOLOGY

Vol. XIX June 1966 No. 2



Published for

THE INDIAN PHYTOPATHOLOGICAL SOCIETY

INDIAN AGRICULTURAL RESEARCH INSTITUTE

NEW DELHI-12 (INDIA)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE OF CONTENTS

PAGES

A comparative study of the resistant and susceptible banana varieties to fungal diseases—*R. Raghunathan, A. Mahadevan and G. Rangaswami* ... 141

Insecticidal control of Nephotettix spp., the vector of Tungro and Yellow dwarf diseases of rice in the Philippines—*V. T. John* ... 150

Variation in the pathogenicity of single-zoospore isolates of Sesamum Phytophthora—*S. P. Sehgal and N. Prasad* ... 155

Fine structure of rust haustoria in susceptible and resistant wheat—*M. S. Manocha* ... 159

Biochemical changes in the banana fruit-coat caused by Gloeosporium musarum infection—*R. Raghunathan, A. Mahadevan and G. Rangaswami* ... 162

Effect of certain vitamins on Sclerotium cepivorum Berk.—*L. K. Joshi and M. P. Haware* ... 168

Studies on the perennation and survival of Sesamum Phytophthora—*S. P. Sehgal and N. Prasad* ... 173

Viability of fungicide-treated wheat and barley seeds in storage—*J. S. Grewal and S. Kapoor* ... 178

Status of linseed-rust races in India and sources of resistance—*D. P. Misra and R. Prasada* ... 184

Studies on a mosaic disease of pea (Pisum sativum L.)—*T. N. Sreenivasan and T. K. Nariani* ... 189

Estimation of losses due to ground-nut mosaic—*V. V. Chenulu, R. L. Munjal, T. S. Hora and Amar Singh* ... 194

Genus Ciliochorella in India—*R. L. Munjal* ... 198

Certain physiologic characteristics of Ustilago avenae and U. kolleri—*Avdhesh Narain* ... 201

Cercospora species causing leafspot of grapes in India—*R. L. Munjal and K. K. Sethi* ... 209

Some aerobiological observations on the rice Stackburn fungus, Trichoconis padwickii—*T. Sreeramulu and B. P. R. Vittal* ... 215

Resistance of paddy varieties and cultures to blast in Chhota Nagpur—*A. K. Dutta and S. M. Ghufran* ... 222

(Contd. on 3rd cover)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A COMPARATIVE STUDY OF THE RESISTANT AND SUSCEPTIBLE BANANA VARIETIES TO FUNGAL DISEASES

R. RAGHUNATHAN, A. MAHADEVAN AND G. RANGASWAMI

(Accepted for publication December 10, 1965)

Banana varieties are attacked by fungal, bacterial and viral diseases (Wardlaw, 1961). The variety "Monthan" (*Musa sapidisiaca*. Jacab) is susceptible to leaf-spot diseases, while another speeies *Musa balbisiana* Colla is resistant (Cheesman and Wardlaw, 1932; Wardlaw, 1939). The culinary variety, Kattu Vazai (*M. balbisiana*), is relatively resistant to leaf-spot diseases. Klein (1959) noted that the youngest leaves of actively growing plants were free from diseases. Rangaswami and Kulandaiswamy (1962) reported that anthracnose infections rarely occurred in the young, green and unripe fruits, while the disease occurred commonly on ripe fruits.

Biochemical differences in the resistant and susceptible plants were reported by Buxton (1962). A variety susceptible to *Fusarium oxysporum* f. *cubense* had no detectable cystine or threonine; while leucine, serine and tyrosine were absent in the resistant variety. Deese and Stahmann (1962) found that the wilt-resistant bananas contained more phenolic compounds than the susceptible plants.

The distribution of amino acids, carbohydrates, organic acids and phenols in the disease-resistant and susceptible banana leaves were investigated. The differences in the occurrence of these compounds during the developmental stages of the leaf were also determined.

MATERIAL AND METHODS : Banana species, *Musa sapidisiaca* Jacob variety Monthan, susceptible to leaf-spot diseases and the resistant variety, Kattu Vazai *Musa balbisiana* Colla were included in these studies. Leaves at three different stages, i.e. young, middle and old, were analyzed.

Analysis of amino acids : Fresh leaves, 30 gm. in weight, were extracted in 80 per cent cold ethyl alcohol by grinding in a pestle and mortar for 10 minutes (Radhakrishnan *et al.*, 1955); the extract was filtered and centrifuged for five minutes at 3000 r.p.m. and the supernatant liquid was used.

Fifty microlitres of the supernatant liquid was spotted on Whatman filter paper No. 1 and developed two-dimensionally in n-butanol-acetic acid-water (9:1:2.2), followed by phenol-water (4:1) for 12 hours. The chromatograms were air-dried, sprayed with ninhydrin (0.5 g. ninhydrin, 95 ml. acetone, 5 ml. water) and dried for 2 hours. Authentic samples were similarly spotted and developed to identify the unknowns.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The amino acids were quantitatively estimated by eluting the spot in 8 ml. of alcohol copper sulphate solution (7.2 ml. of 75 per cent ethanol and 0.8 ml. of 0.1 per cent copper sulphate) for 2 hours and read in a Klett-Summerson colorimeter, employing a green filter (Demetriades, 1956).

Carbohydrate Analysis : Carbohydrate from the leaf was extracted according to the method of Lulla and Johar (1954) and chromatographically separated in the following manner. Twenty microlitres of the extract was spotted on Whatman filter paper No. 1 and ascendingly developed in n-butanol-acetic acid-water (4:1:5-organic phase) for 12 hours. The chromatograms were sprayed with benzidine reagent and dried in an oven at 70°C. for 10-15 minutes. Upon them, yellow to brown spots appeared.

The spots were cut and eluted in 20 ml. of distilled water for 15 minutes and filtered. Aqueous phenol (0.1 ml. of 80 per cent) and conc. sulphuric acid (5.6 ml.) were added, the mixture was cooled and the orange-yellow colour was read in a Klett-Summerson colorimeter, using a blue filter (Sims and Christensen, 1962).

Organic acids in the alcohol extract were determined by spotting 50 microlitres on Whatman filter paper No. 1 and developing ascendingly in n-butanol-formic acid-water (6:1:1) for 10-12 hours. Bromophenol blue was sprayed to detect the organic acids.

For quantitative determination, the spots were cut, placed in 10 ml. of distilled water for 10 minutes and titrated against N/500 NaOH with phenolphthalein indicator.

Estimation of phenols : Twenty-five-gram leaf samples were ground in a pestle and mortar with two volumes of acetone, and the mixture was stored for 2 to 3 days at room temperature, filtered, and the filtrates were concentrated *in vacuo* to 3 ml.

Fifty microlitres of the concentrated extract was spotted on Whatman filter paper No. 1 and developed ascendingly in n-butanol-acetic acid-water (4:1:2.2), followed by 2 per cent acetic acid. The paper was run for 7 to 9 hours in the first solvent and 2 hours in the second solvent. The dried chromatograms were sprayed with diazotized sulphanilic acid (Kuc *et al.*, 1956).

Total phenols were estimated by employing Folin-Denis reagent (Bray and Thorpe, 1954). An aliquot of 100 microlitres of the extract was taken in a boiling tube and made up to 10 ml. with distilled water. One ml. of Folin-Denis reagent and two ml. of saturated sodium carbonate were added. The tube was heated for one minute in a boiling-water bath and cooled in running water. The blue solution was diluted to 25 ml. with water and its absorbancy was read in a Klett-Summerson colorimeter, employing a red filter. The total phenol was expressed in catechol equivalent.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Carbon-nitrogen ratios of banana leaves : Young, middle and old banana leaves were cut into small pieces and air-dried. Each sample was ground to a fine power and the carbon was estimated by Walkley and Black's method (1958), and nitrogen by the method of Bremner (1960).

RESULTS : Resistant and susceptible banana leaves exhibited some biochemical differences in the distribution of amino acids, sugars, organic acids and phenols, both in quantity and quality.

The leaves of Monthan at the three stages contained the following amino acids : leucin(s), valine, proline, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine, lysine and cystine. On the other hand, young leaves of Kattu Vazai contained valine, tryptophan, tyrosine, leucin(s), alanine, threonine, glycine, serine, glutamic acid, histidine, arginine and lysine, while threonine was absent in the middle leaves Old leaves contained valine, tryptophan, alanine, glycine, serine, glutamic acid, aspartic acid and histidine.

The amount of amino acids fluctuated quantitatively in both the varieties (Table 1). In Monthan, leucine, valine, proline, tyrosine, threonine, glycine, lysine and cystine concentrations decreased with increasing age. Serine and aspartic acid decreased in concentrations from young to middle and increased in the old leaf. Arginine increased from the young to middle and then decreased, whereas alanine decreased from young to middle and increased in the old leaf. In Kattu Vazai, the concentrations of valine, tryptophan, alanine, glycine, serine and glutamic acid increased from young to middle and then decreased in the old leaf, whereas leucin(s) and tyrosine concentrations increased from the young to the middle leaf and disappeared in the old leaf. Arginine and lysine decreased from the young to the middle leaves and were absent in the old leaves. Aspartic acid was present in old tissues, while histidine decreased from young to old tissues.

The carbohydrate contents in the susceptible and resistant species also differed. Monthan leaves contained fructose, glucose and sucrose ; the fructose concentration decreased from the young to the middle leaf and disappeared completely in the old leaf. Similarly, glucose and sucrose decreased from the young to the old leaf. In Kattu Vazai, fructose, glucose and sucrose were found in the various stages of leaf-growth however, the concentration decreased with maturity.

The occurrence of organic acids in the banana varieties also differed. Succinic acid was detected in the middle and the old Monthan leaves, while it was absent in the young leaves. Malic acid decreased from the young to the middle leaf and again reached a maximum in the old leaves. In Kattu Vazai, malic and citric acids were present and malic acid decreased with the age of the leaf, whereas citric acid concentrations fluctuated.

The distribution of phenolic compounds in leaves revealed some significant differences between the resistant and susceptible varieties. The susceptible "Monthan" leaves had five positive spots in the young and

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

middle leaves, whereas only four phenols occurred in the old leaves. Young leaves of Kattu Vazai contained seven phenols, which decreased to six in the middle and to five in the old leaves.

The amount of total phenols decreased with the age of the leaf in both the varieties (Table 2). The resistant variety contained more phenols than the susceptible variety.

TABLE 1 : Concentrations of free amino acids in the banana leaves at three different stages of development.

Amino acid	Amino-acid content in mg/g of leaf on oven-dry basis					
	Young leaf		Middle leaf		Old leaf	
	Monthan	Kattu vazai	Monthan	Kattu vazai	Monthan	Kattu vazai
Lucin (s)	8.28	1.34	10.34	3.42	2.93	0.00
Valine	14.42	2.49	8 74	4.56	4.79	1.89
Proline	14.16	0.00	9.82	0.00	6.93	0.00
Tryptophan	0.00	3.46	0.00	4.55	0.00	2.32
Tyrosine	14.46	3.89	10.46	8.67	7.13	0.00
Alanine	25.15	6.08	22.11	8.48	27.71	5.78
Threonine	29.21	1.78	20.49	0.00	15.38	0.00
Glycine	14.15	3.86	12.63	7.36	8.40	3.24
Serine	30.39	6.39	15.74	12.78	24.23	3.4
Glutamic acid	13.49	2.64	2.14	5.30	0.42	2.18
Aspartic acid	33.32	9.00	19.57	0.00	25.35	1.12
Histidine	0.00	19.63	0.00	3.95	0.00	2.87
Arginine	12.37	0.84	13.01	0.61	6.94	0.00
Lysine	5.00	1.99	4.89	1.56	1.36	0.00
Cystine	28.94	0.00	9.78	0.00	3.48	0.00

TABLE 2 : Total phenols in the banana leaves at different stages of development.

Age of the leaf	Total phenols (catechol equivalent) in mgm.	
	Monthan	Kattu Vazai
Young leaf	1.758	2.99
Middle leaf	0.858	1.32
Old leaf	0.642	1.02

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The carbon and nitrogen ratios differed considerably in these varieties. The ratio was higher in the resistant than in the susceptible variety (Table 3). A general increase in the C/N ratio was observed with leaf maturity.

TABLE 3 : Carbon-nitrogen ratio of Monthan and Kattu Vazai leaves

Age of the leaf	Carbon-Nitrogen ratio	
	Monthan	Kattu Vazai
Young leaf	16:1	23:1
Middle leaf	18:1	26:1
Old leaf	19:1	27:1

DISCUSSION : The varieties Kattu Vazai (*Musa bulbisiana*) is resistant to leaf-spot diseases caused by *Cercospora musae* and *Helminthosporium gibberosporium*, while Monthan (*M. sapidisiaca*) is susceptible to both the diseases (Rangaswami and Kulandaiswamy, 1962). The relative resistance of *M. balbisiana* to fungal diseases might be attributed to the differences in the distribution of amino acids, carbohydrates, including organic acids, phenols and C/N ratios.

Amino acids in Monthan leaves decreased during maturity. Both glutamic acid and cystine declined in the old tissues, while their concentration was high in the young tissues. Rangaswami and Natrajan (1965) also reported a similar situation in banana species. Lakshminarayanan (1965) showed that sulphur amino acids occurred consistently in the wilt-resistant cotton species. Papavizas and Davey (1963) noted that sulphur amino acids inhibited *Aphanomyces* root rot of peas. Mahadevan (1965) showed that cystine and cysteine inhibited the *in vitro* development of *Fusarium oxysporum* f. *vasinfectum*.

It was found that the young leaves of Kattu Vazai possessed large amounts of histidine and tryptophan. Hrushovetz (1957) reported that the virulence of *Helminthosporium sativum* decreased in the presence of 0.1 per cent histidine and tryptophan. Van Andel (1958) showed that histidine increased plant resistance to scab infection. Rust development in excised wheat leaves was inhibited by histidine (Samborski and Forsyth 1960). The importance of tryptophan in increasing resistance in many plants against parasitic attacks has been reported (Johan *et al.*, 1960 ; Orellana and Thomas, 1962).

The resistant species, Kattu Vazai, contained more sugars than in the subsceptible Monthan. Barnett and Lilly (1958) noted an increase resistance with increased sugar concentrations, while Llyes *et al.*, (1959) reported that rust-resistant wheat contained higher sugar levels than the susceptible varieties.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phenolic compounds in the resistant and susceptible plants exhibited some distinct relationships. Kattu Vazai contained more phenols than the susceptible species. However, in both the species, more phenols occurred in the young tissues, in which they decreased with the increasing age.

The increased quantity of phenols in the young tissues of both susceptible and resistant plants offered protection against parasitic attacks. Resistant tissues possessed more phenols than the tissues of the susceptible variety. Interestingly, the resistant varieties contained 7 DSA spots, while the susceptible variety had only 5 spots. The nature of these phenolic compounds and their individual effects upon the fungal development should receive further attention.

The influence of nitrogen on the phenolic metabolism should be considered in disease-resistance. Kirkham (1954) suggested that high amino nitrogen in the medium completely degraded aromatic compounds by the exogenous activity of fungal polyphenol oxidase. An optimum ratio of amino acids to total phenols is important in making the host either susceptible or resistant. High amino acids resulted in the breakdown of resistance to fungal infections, whereas lesser amounts may act favourably with phenols in the Kattu Vazai leaves, exhibiting resistance to leaf-spot diseases. Kirkham (1954) and Flood and Kirkham (1960) suggested similar mechanisms in apple and pear varieties.

From our study, it appears that the disease-resistance mechanism in banana may involve an interaction of amino acids and phenols. Future studies on the interaction of these factors should receive attention in infection studies.

Summary

The amino acid, carbohydrate, organic acid and phenol contents of the leaves of two banana varieties, Monthan (*Musa sapidisiaca* Jacob), highly susceptible to many fungal infections, and Kattu Vazai (*M. balbisiana* Colla), highly resistant to most fungal infections, were compared by chromatographic assays. For this purpose, young, middle and old leaves of the plants were carefully selected and examined. The leaves of Monthan contained the following amino acids at all the three age levels: leucin, valine, proline, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine, lysine and cystine. In Kattu Vazai, the young leaves contained valine, tryptophan, tryrosine, leucin, alanine, threonine, glycine, serine, glutamic acid, histidine, arginine and lysine, whereas threonine was absent in the middle leaves, and in the old leaves tyrosine, lysine, threonine and leucine were absent but aspartic acid was present in addition. Both Monthan and Kattu Vazai leaves contained fructose, glucose and sucrose and their concentrations fluctuated with maturity. Succinic, malic and citric acids were noticed in Monthan leaves, while Kattu Vazai contained malic and citric acids only. Five phenols were detected in the susceptible variety, while the resistant plant contained seven phenols, and their concentrations decreased in both the varieties

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

with increasing age. The carbon-nitrogen ratio was higher in the resistant plants than in the susceptible ones. The importance of these biochemical differences between the susceptible and resistant varieties is discussed.

Annamalai University, Annamalainagar,
Madras State

References

VAN ANDEL, M. O. (1958) Investigations on plant chemotherapy. II. Influence of amino acids on the relation plant pathogen. Tijdschr. Planthenziekten **64** : 307-327.

BARNETT, H. L., and V. G. LILLY (1958) The use of fungus parasites in basic studies of parasitism. Proc. West Virginia Acad. Sci **30** : 134 (abst.).

BRAY, G. G. and W. V. THORPE. (1954) Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. **1** : 27-52.

BREMNER, J. M. (1960) Determination of nitrogen in soil by Kjeldahl method. *J. Ag. Sci.* **55** : 11-33.

BUXTON, E. W. (1962) Root exudates from banana and their relationship to strains of *Fusarium* causing Panama wilt. *Ann. Appl. Biol.* **50** : 269-282.

CHEESMAN, E. E. and C. W. WARDLAW (1937) Specific and varietal susceptibility of bananas to *Cercospora* leaf spot. *Trop. Agricul.* **14** : 335-336.

DEESE, D. C. AND M. A. STAHMANN. (1962) Pectic enzymes and cellulase formation by *Fusarium oxysporum* f. *cubense* on stem tissues from resistant and susceptible banana plants. *Phytopathology* **52** : 247-255.

DEMETRIADES, S. D. (1956). Chromatographic detection of free amino acids in normal and iron-deficient plants of *Hibiscus esculentus*. *Nature* **177** : 95-98.

FLOOD, A. E. AND D. S. KIRKHAM (1960) The effect of some phenolic compounds on the growth and sporulation of two *Venturia* spp. in "Phenolics in plants in health and disease". *Pergamon Press, London.*

HRUSHOVETZ, S. B. (1954) The effect of infection by *Helminthosporium* sativum on the amino acid content of wheat roots. *Can. J. Botany* **32** : 571-575.

JOHAM, H. E., L. S. BIRD AND C. W. BLACKMAN, (1960) Amino acids in cotton varieties resistant and susceptible to *Xanthomonas malvacearum*. *Proc. Assoc. 8th Agric. Workers* **57** ; 244-247.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

KIRKHAM, D. S. (1954) Significance of the ratio between water soluble aromatic nitrogen constitutents of apple and pear in the host-parasite relationship of *Venturia* sp. *Nature* **173** : 690-691.

KLEIN, H. H. (1959) United Fruit Company Research Briefs. No. 12.

KUC, J., R.E. HENZE, A.J. ULLSTRUP AND F.W. QUACKENBUSH (1956). Chlorogenic and Caffeic acids as fungitoxic agents produced by potatoes in response to inoculations with *Helminthosporium carbonum*. *J. Am. Chem. Soc.* **78** : 3123-3125.

LAKSHMINARAYANAN, K. (1955) Biochemical aspects of vascular wilts. Symposium on soil microorganisms and plant well being. *Proc. Indian Acad. Sci*, **41(B)** : 92-154.

LLYES, W.E., M.C. FUTRELL AND I.M. ATKINS. (1959) Relation between reactions to race 158 of stem rust and reducing sugars and sucrose in wheat. *Phytopathology* **49** : 254-256.

LULLA, B.S. AND D.S. JOHAR. (1954) Organic acids in bananas. *Curr. Sci.* **23** : 362.

MAHADEVAN, A. (1965). Biochemistry of Fusarium wilt resistance in cotton. I. The influence of amino acids on fungal growth. *Ind. Phytopath.* (In press).

PAPAVIZAS, G.C. AND C.B. DAVEY, (1962) Prevention of *Aphanomyces* root rot of peas by amino compounds. *Phytopathology* **52** : 24 (abst).

ORELLANA R.G. AND C.A. THOMAS, (1962) Nature of predisposition of castor beans to *Botrytis*. I. Relation of leachable sugar and certain other biochemical constitutents to varietal susceptibility. *Phytopathology* **52** : 533-538.

RADAKRISHNAN, A. N., C. S. VAIDYANATHAN, AND K.V. GIRI. (1955) Nitrogenous constituents in plants. I. Free amino acids in leaves and leguminous seeds. *J. Ind. Inst. Sci. Sect. A-B.* 37 : 178.

RANGASWAMI, G. AND S. KULANDAISWAMY. (1952). Studies on the relative susceptibility of banana varieties to three leaf spot diseases. *J. Annamalai Univ.* **23(b)** : 127-134.

RANGASWAMI G. AND S. NATARAJAN (1964) Studies on some chemical components of banana leaves and fruits as influenced by fungal infections. *Phytopathology* (in press).

SAMBORSKI, D.J. AND F.R. FORSYTH. (1960) Inhibition of rust development on detached wheat leaves by metabolites, antimetabolites and enzyme poisons. *Can. J. Botany* **38** : 467-476.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SIM, K. AND C.M. CHRISTENSEN (1962) Quantitative analysis of sugars in Royal jelly. *Nature* **196** : 1208-1209.

WALKLEY A. AND. I.A. BLACK. (1958) in Soil Chemical Analysis, edited by J.L. Jackson, *Prentice Hall Inc.*, England.

WARDLAW, C.W. (1939) *Cercospora* leaf spot of bananas *Nature* **144** : 11-14.

WARDLAW 1961. Banana Diseases. *Longmans Green and Co., Ltd.*, London.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

INSECTICIDAL CONTROL OF NEPHOTETTIX SPP., THE VECTOR OF TUNGRO AND YELLOW DWARF DISEASES OF RICE IN THE PHILIPPINES

V. T. JOHN[1]

(Accepted for publication November 25, 1965)

INTRODUCTION : In recent years, leafhopper-transmitted virus diseases of rice are causing serious economic losses in many S.-E. Asian countries. In spite of the seriousness of this problem, very little work has been done on the control of these virus diseases. The incidence of hoja blanca has been greatly reduced by the introduction of disease-resistant varieties (Beachell *et al.*, 1959). The biological control of vectors has promising protentialities but needs elaborate studies and is time-consuming. Nevertheless, as a quick measure of control, clean cultural practices, the proper timing of sowing and the use of safe and efficient insecticides may be tried. Indeed, the advent of systemic insecticides opens up a fruitful field of research for the control of many vectors. The present study is aimed at selecting a proper insecticide to control the green rice leafhopper, *Nephotettix* spp., the vector of tungro and yellow dwarf diseases of rice in the Philippines.

MATERIAL AND METHODS : Taichung Native 1 rice seedlings were used throughout the study, since this variety is very susceptible to the two diseases. The seedlings were transplanted to 4½″ clay pots (one seedling/pot) 15 days after germination, The pots were coated inside with 'alkatran' (coal-tar) and contained 450 g. soil. Three days after transplanting, the insecticidal treatments were given. In all cases, 24 hours after the addition of insecticides, the test-insects were caged. Wild populations of *Nephotettix* spp. were collected from field rice-nurseries by sweeping, and adults were picked by an aspirator. These were then maintained on large Taichung N_1 plants in suitable cages. Most of the population consisted of *N. impicticeps* Ishihara and a few of *N. apicalis* Motch. A high percentage of insects died of sweeping injuries and only those surviving 3 days after collection were used for tests.

The test-cages were 10″ high and 3″ in diameter and were made up of 'Mylar' film with two air-vents covered by nylon net and a close-fitting lid which facilitated the opening of the cages to let in the insects. The basal open portion of the cage was plunged into the soil. Each cage contained one plant and 10 insects and for each insecticide 10 plants were used. All the experiments were repeated thrice and the values (mean 300 insects) were statistically analysed.

EXPERIMENTAL : *Soil treatment* : The insecticides used for soil treatment were (1) ∝-BHC (liquid form) (2) dimecron (liquid form)

[1]Present address : N.R.C. Fellow, National Research Council, Research Station, 6660, N.W. Marine Dr., Vancouver 8, B.C., Canada.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(3) sevin (solid) and (4) thimet (solid). All these insecticides were added to the soil at the rate of 3 kg. active material/ha. around the plant 3 days after transplanting. Prior to the caging of the insects, a fresh layer of dry soil was added to the soil to prevent the insects from contacting the insecticide when they are caged. Mortality counts were made 2, 4, 6, 8 and 24 hours after caging. The results are presented in Table 1.

TABLE 1 : Percentage mortality of *Nephotettix spp.* owing to soil treatment with insecticides (mean of 300 insects).

Hours after caging	Control	8-BHC	Dimecron	Sevin	Thimet
2	2.00	3.00	32.67	28.67	13.00
4	6.33	5.00	91.33	68.67	56.67
6	8.33	6.00	96.00	85.33	80.33
8	8.33	8.00	98.67	89.67	89.00
24	11.62	15.33	99.67	96 00	99.33
T means	7.33	7.13	83.67	74.33	67.67

LSD (Treatment) = 6.84

It is evident that 8-BHC is not effective against *Nephotettix* spp., whereas dimecron, sevin and thimet are capable of inducing a high mortality, 4-8 hours after the caging of insects. Dimecron seems to have a quicker action than thimet or sevin.

Residual effect of dimecron, sevin and thimet : To study the residual effect, the insects were caged 5, 10, 15, 20, 25 and 30 days after the addition of insecticides and counts taken 24 hours after caging. The results are presented in Table 2.

It is clear from Table 2 that none of the 3 insecticides induces a 100 per cent mortality 5 days after application. To induce a 40 per cent mortality (this value is chosen arbitrarily because about 40 per cent of these insects in nature are viruliferous) dimecron is effective up to 5-10 days, sevin up to 15-20 days and thimet up to 25-30 days.

TABLE 2 : Residual effect of dimecron, sevin and thimet in per cent mortality of *Nephotettix* spp. (mean of 300 insects).

Days after insecticidal application	Control	Dimecron	Sevin	Thimet
5	0	62	78	81
10	15	28	45	88
15	6	14	49	87
20	13	15	40	60
25	20	13	16	52
30	36	12	14	33

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Spray treatment : The insecticides used for spray treatment were (1) malathion (liquid), at the rate of 3 ml/gallon, (2) endrin (liquid) at the rate of 7 ml/gallon (3) sevin (solid) at the rate of 4.5 g/gallon and (4) DDT (solid) at the rate of 4.5 g/gallon. Aqueous solutions of these chemicals were sprayed on to the aerial parts of plants 3 days after transplanting. Control plants were sprayed with distilled water. Twenty-four hours later the test-insects were caged. Mortality counts were made 2, 4, 6, 8 and 24 hours after caging. The results are presented in Table 3.

TABLE 3 : Percentage mortality of *Nephotettix* spp. on Taichnung N_1 sprayed with various insecticides.

Hours after caging	Control	Malathion	Endrin	DDT	Sevin
2	1.3	52.0	4.6	11.6	52.6
4	4.6	83.3	12.0	26.6	78.6
6	7.3	89.3	20.3	34.6	89.6
8	9.6	91.6	24.6	41.3	92.0
24	39.0	98.6	59.0	77.3	98.6
T means	11.0	82.7	24.1	38.3	82.3

LSD (Treatment) = 8.69

It is evident from the above Table that malathion and sevin are identical in their action and are far superior to endrin and DDT. DDT, however, seems to be superior to endrin.

Residual effect of sprayed malathion and sevin : The residual effect of malathion and sevin was studied 5 and 10 days after spraying and the results are presented in Table 4.

TABLE 4 : Residual effect in percentage mortality of malathion and sevin sprayed on the leaves of Taichung N_1.

Days after spray	Control	Malathion	Sevin
5	31.0	27.0	42.0
10	11.0	19.0	10.0

Both malathion and sevin lose their effect by about the 5th day after spraying to cause a 40 per cent mortality (Table 4).

DISCUSSION : Screening studies for the evaluation of insecticides can often be misleading. For instance, Bang and Kae (1963) reported a study of a similar nature, where the test-insects were collected from the field by sweeping and were *immediately* introduced into the test-cage for studying the insecticide-induced mortality. In the present work, more than 50 per cent of the insects collected by sweeping died because of mere sweeping injuries, if they are caged on healthy plants. Therefore, a time lag should be given before these insects are used for insecticidal test.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Adequate replication is essential to arrive at a proper choice of the insecticide. In the study of Bang and Kae (1963) the number of test-insects apparently was inadequate. In the present work, however, attention has been given to replication and very good agreement has been found between replicates.

Among the chemicals tested in this study, thimet has very powerful insecticidal properties with a long residual effect, but its extremely high mammalian toxicity becomes a limiting factor in its use. Sevin, when used as a soil insecticide, seems to be promising for controlling *Nephotettix* spp., not only from the results reported here, but also from our field experiments where extremely high yields were obtained in sevin-treated plots (unpublished data). On the other hand, sevin used as a foliar spray, although effective for a short period, becomes ineffective by the fifth day (Table 4). Furthermore, unlike thimet, sevin has a low mammalian toxicity (West and Hardy, 1961).

The present study does not presuppose that the soil insecticides used are true systemics. It is obvious from Table 1 that 8-BHC is very ineffective in controlling the vector although it is claimed to be effective against *Sogata orizicola* Muir, the vector of hoja blanca (McGuire *et al.*, 1960), *Delphacodes striatella* Fallen, the vector of rice stripe (Hashioka, 1951) and rice stem borers (Pathak, personal communication).

Summary

Critically controlled experiments show that sevin (1-naphthyl-N-methyl-carbamate) is a good soil insecticide having residual effect for 15-20 days when applied at the rate of 3 kg. active material/ha. in controlling *Nephotettix* spp. Among spray insecticides malathion and sevin are promising but their effect is lost within 5 days. Endrin and 8-BHC are not effective against *Nephotettix* spp.

Acknowledgements : The author is indebted to The International Rice Research Institute, Laguna, Phillippines, for the facilities and to the Ford Foundation for financial support for carrying out this project. To Drs. M D. Pathak, Entomologist and B.T. Onate, Statistician of the above institute, for their keen interest and help, I owe my deep sense of gratitude.

International Rice Research Institute

Laguna, The Phillippines

References

Bang Y.H. and B.M. Kae (1963) The green rice leafhopper, *Nephotettix bipunctatus cincticeps* and its control in Korea. *J. econ. Entom.*, **56** : 773-776.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BEACHELL H.M., N.E. JODON, T.H. JOHNSTON, J.R. THYSELL, J.G. ATKINS AND H.A. LAMEY (1959). Breeding rice varieties to the virus disease hoja blanca. *News Lett. Int. Rice Comm.* **8** : 6-9.

HASHIOKA, Y. (1951) Rice stripe (Oryza virus 2). *Rev. appl. Mycol.*, **30** : 539.

MCGUIRE J.U., W.M. MCMILLAN AND H.A. LAMEY (1960) Hoja blanca disease of rice and its insect vector. *Rice J.*, **63** : 15-16.

WEST, T. F. AND E. J. HARDY (1961) Chemical Control of Insects. Chapman and Hall Ltd., London.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

VARIATION IN THE PATHOGENICITY OF SINGLE-ZOOSPORE ISOLATES OF SESAMUM PHYTOPHTHORA*

S. P. SEHGAL AND N. PRASAD

(Accepted for publication January 10, 1966)

Among the oilseed crops of India, sesamum (*Sesamum orientale* L.) is one of the most important. The crop suffers serious losses due to a blight caused by *Phytophthora parasitica* var. *sesami* Prasad. The fungus attacks plants at all ages. Nolla (1928) and Powers and Lucas (1952) observed both weakly and highly pathogenic strains in *P. parasitica* var. *nicotianae*, causing the black shank disease of tobacco, the former attributing the weak pathogenicity to old age of the culture. Apple (1954, 1957) classified isolates of the black shank fungus in three pathogenic classes arbitrarily based on their virulence on young and adult plants. He observed great variablity in isolates, ranging from highly pathogenic even on resistant varieties to non-pathogenic on susceptible ones. Gemawat (1956) reported that isolations made from different field specimens showed wide variations in virulence in various isolates of *P. parasitica* var. *sesami*. In the present studies, during the course of pathogenicity tests with different single-zoospore isolates of *Phytophthora* attacking sesamum, marked variation was noticed in the symptoms produced on seedlings and adult plants by morphologically and physiologically similar isolates and the results are presented in this paper.

MATERIAL AND METHODS : Collections of diseased samples were made from all over Rajasthan and isolations of the fungus were made from the freshly collected samples only. Single-zoospore cultures were prepared by the following method: A small piece of mycelium was floated in sterilized tap-water for about 36 hours for inducing the fungus to produce a large number of zoosporangia. From the zoosporangia-producing mycelium, a little piece of mycelium bearing abundant zoosporangia was picked up with the help of sterilized needles and was placed on a clean slide along with a drop of sterilized tap-water to further induce zoospore formation within the zoosporangia. A few drops of water were added regularly on the slide. A large number of zoospores were liberated in water on the slide within 15 minutes. More drops of sterilized water were poured on the slide to dilute the concentration of zoospores. From such a zoospore suspension, a small drop was spread over plain difco-agar plates with the help of a clean sterilized glass rod. Immediately after placing the drop of water on agar, the zoospores showed rapid circular jerking movements but soon (within 10-15 minutes) came

*Based on the thesis submitted by the senior author and approved for the Ph. D. degree by the Rajasthan University.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

to rest. Such plates were examined under the low power of a microscope and single zoospores were then selected and with the help of sterilized needles transferred to oat-meal-agar slants. Visible growth of the fungus occurred on slants after 6 days at room temperature (20° to 30° C). In all studies, only cultures obtained as a result of picking up single zoospores were used.

Local sesamum seeds were grown in sterilized soil in earthen pots 9″ × 12″ in size. Two trials, i.e. one with 18-day-old seedlings and the other with 2-month-old plants were made in green house.

Inoculations in both the trials were made from actively growing ten-day old cultures by removing a little soil from the base of each plant, and placing a uniform amount of the fungus culture along with a little medium near the base of the stem and covering again with soil. The plants were watered heavily three to five times every day to provide favourable humid conditions for successful infection. The experiments were conducted during the months of July, August and September.

The reactions of various isolates on host plants were graded as follows :

1. Seedling stage trial on 28-day-old plants.

(*a*) Symptoms appearing within 48-96 hours after inoculation and followed by complete collapse of plants within 10 days : Highly pathogenic (Group I).

(*b*) Symptoms appearing within 7 days on all the above-ground portions, *i.e.* stem, leaf, etc. but the necrosis of plants not occuring within 10 days after inoculation : Pathogenic (Group II).

(*c*) Symptoms appearing on leaves and petioles within 10 days and resulting in either leaf-fall or rotting of leaves : Moderately pathogenic (Group III).

(*d*) Symptoms appearing on leaves and in a mild form after 10 days : Weakly pathogenic (Group IV).

(*e*) Symptoms absent : Non-pathogenic (Group V).

2. Adult stage trial : (Lesion length) on 2-month-old plants.

(*a*) Total length of lesion 16 cm. or above : Highly pathogenic (Group I).

(*b*) Total length of lesion between 11-15 cm. : Pathogenic (Group II).

(*c*) Total length of lesion between 6-10 cm. : Moderately pathogenic (Group III).

(*d*) Total length of lesion 5 cm. or below : Weakly pathogenic (Group IV).

(*e*) Symptoms absent : Non-pathogenic (Group V).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL : During the initial pathogenicity tests for the study of variability within the fungus, 98 single-zoospore isolates were tested repeatedly on 28-day-old sesamum plants. Out of 98 isolates, 9 showed a high degree of pathogenicity ; 28 proved pathogenic, 20 moderately pathogenic, 10 weakly pathogenic and 31 proved non-pathogenic.

In Table 1, the behaviour of a few selected isolates which represented collections from all regions of Rajasthan is given. These tests were made repeatedly with both the seedling and the adult plants in the year 1960.

TABLE 1 : Observations on the virulence of different isolates on young and adult plants.

S. No.	Isolate No. and place of collection	28-day-old plants showing pathogenic grade	Two-month-old plants showing the length of lesion in cm. after (in days) 7	14	21	Pathogenic grade
1.	2-Dabok	I	5	11	18	I
2.	22-Nathdwara	II	6.5	10	15	II
3.	26-Nathdwara	III	2	5	8	III
4.	40-Khamnore	IV	—	2	4.5	IV
5.	55.Udaipur	IV	—	3	5	IV
6.	57-Pali	III	3.5	6	10	III
7.	69-Pali	II	5	8	14	II
8.	77-Kota	II	4	8	15	II
9.	85-Deoli	I	5	10	16	I
10.	93-Banswara	II	3	9	13	II
11.	94-Kalingra	II	5	10	13	II
12.	37-Khamnore	V	—	—	—	V
13.	65-Pali	V	—	—	—	V

Results indicate that both non-pathogenic and pathogenic (with variation in pathogenicity) isolates occur in nature in one and the same locality.

DISCUSSION : Variation in pathogenicity in the single-spore isolates of a fungus are commonly found. Hansen (1938, 1942) reported that many fungi occur in nature in a heterocaryotic condition and when such fungi are studied with single-spore cultures, great variability appears in different isolates. Apple (1957) observed variations in the virulence in single-zoospore isolates of *P. parasitica* var. *nicotianae*. He further reported that on re-single-zoosporing of cultures, more pathogenic and cultural variations were observed. In single-zoospore isolates of sesamum *Phytophthora* too, variations in pathogenicity of different isolates were found, which ranged from highly pathogenic to non-pathogenic. Apple (1954) reported in the case of the black shank disease of tobacco that virulent and mild strains of *P. parasitica* var. *nicotianae* were confined to severely diseased areas and mild diseased areas respectively, but in the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

case of the isolates of *Phytophthora* attacking sesamum, all isolates showing different grades of virulence were found in one and the same locality and all such strains were distributed evenly in all the sesamum-growing areas of Rajasthan representing varied types of climate, *i.e.* arid (Deoli), semiarid (Pali) and wet (Banswara). Therefore it is reasonable to conclude that severity of the disease in humid region was due to the presence of favourable conditions of moisture and temperature and not due exclusively to the presence of virulent strains.

SUMMARY

In nature many pathogenic variants of the blight fungus are found; these isolates do not show any morphological or physiological dissimilarity. All the highly pathogenic and non-pathogenic isolates are not confined to some definite locality but all are uniformly distributed in all the regions.

ACKNOWLEDGEMENTS : The authors are grateful to the Director of Agriculture, Rajasthan, and Dr. R. L. Mathur, Plant Pathologist, Rajasthan, for facilities.

Plant Pathology Section
Sub Station, Kota
Rajasthan

REFERENCES

APPLE, J. L. (1954) Variation in pathogenicity of *Phytophthora parasitica* var. *nicotianae*. *Plant Dis. Rept.* **38** : 774-776.

——— (1957) Pathogenic, cultural and physiological variation within *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* **47** : 733-740.

GEMAWAT. P. D. (1956) Further studies on *Phytophthora* blight of sesamum. *M. Sc. (Agri.) Thesis, Gujarat University.*

HANSEN, H. N. (1938) The dual phenomenon in imperfect fungi. *Mycologia* **30** : 442-455.

——— (1942) Heterocaryosis and variability. *Phytopathology* **32** : 639-640.

NOLLA, J.A.B. (1928) The black shank of tobacco in Porto Rico. *J. Dept. Agr. Porto Rico* **7** : 185-215.

POWERS, H. R. (JR) AND G. B. LUCAS (1952) Variation within *Phytophthora parasitica* var. *nicotianae*. *Plant Dis. Rept.* **36** : 335-336.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

FINE STRUCTURE OF RUST HAUSTORIA IN SUSCEPTIBLE AND RESISTANT WHEAT

M. S. MANOCHA*

(Accepted for publication March 9, 1966)

INTRODUCTION : Although electron microscopy of fungi has drawn cosiderable attention during recent years (Hawker, 1965), the understanding of host-parasite relationships at cellular levels is still at its initial stages. A few accounts of fungal haustoria now available are chiefly concerned with the fine structure of the host-parasite interface. Ehrlich and Ehrlich (1963) were the first to show with certainty the presence of a sheath around the haustorium of stem rust of wheat. They called this sheath 'encapsulation' and considered it as an extrusion of haustorium. Peyton and Bowen (1963) and Berlin and Bowen (1964), working on downy mildew, however, interpreted this zone as nuritional in function and attributed its origin to the host cell.

The fine structure of the haustoria of stem rust of wheat on susceptible and resistant host varieties is compared in this report and a possible explanation is presented on the nature and the role of encapsulation.

MATERIAL AND METHODS : The first-leaves of seven-day-old seedlings of Little Club and Khapli wheats were inoculated with *Puccinia graminis* var. *tritici* Eriks. and Henn. race 15B. Rust-infected tissues were cut in 1mm^3 pieces, fixed in 2 per cent $KMNO_4$ for 30 min. at room temperature, dehydrated through Durcupan A (Fluka AG, Buchs, Switzerland) and were embedded in Araldite. Thin sections cut on Porter-Blum microtome with glass knives, were examined in Electron Microscope Phillips 100B, after staining with lead acetate (Milloning 1961).

RESULTS AND DISCUSSION : The fine structure of haustoria in susceptible (Little Club, Figs. 1 and 2) and resistant (Khapli, Fig. 3) hosts appears identical except for a few details. The haustoria are characterized by dense cytoplasm delimited by a well-differentiated wall. The endoplasmic reticulum is extensively developed and consists of elongated cisternae often forming a reticulum. There are numerous mitochondria of variable shapes and sizes with more or less parallel array of cristae. The nuclei are distinct and delineated by a double membrane interrupted by pores (arrows). The haustoria are dicaryotic like other cells of the uredial stage but the micrographs show only a caryon. The haustoria are surrounded by elongated strands of host endoplasmic reticulum (ER) and the stimulated production of host endoplasmic reticulum near or around the haustorium has also been described by Peyton and Bowen (1963). The examination of several micrographs of haustoria in susceptible and

*Present Address : Division of Biosciences, National Research Council, Sussex Drive, Ottawa, Ontario, Canada.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

resistant host cells did not show quantitative difference in mitochondria or endoplasmic reticulum, as pointed out earlier by Ehrlich and Ehrlich (1962).

Figs. 1 and 3 are prepared from infected tissue at six days after inoculation but they show different stages of rust development. The former shows host plasma membrane (PM) closely adhering on the outside of the haustorial wall (fw), whereas the latter shows a well-developed zone (X), known as encapsulation, separating the host plasma membrane from the haustorial wall. It is likely that this zone might have developed as early as 2-4 days after inoculation in the resistant variety. The encapsulation develops 8-10 days after inoculation in the susceptible variety and it corresponds with sporogenesis under experimental conditions. Vesicles (V) produced by Golgi bodies (G) and endoplasmic reticulum in host cytoplasm (Figs. 2 and 3) possibly play a role in the development of encapsulation by discharging their contents at the outer surface of the host plasma membrane. No actual discharge is shown in these micrographs. Channel-like connections in the haustorial wall, as reported by Ehrlich and Ehrlich (1963), were not observed.

The earlier formation of encapsulation in the resistant variety and a definite delay in its development in the susceptible variety is quite indicative of this zone as a defensive barricade put up by the host cell, rather than a nutritional zone. Furthermore, Peyton and Bowen (1963) have clearly pointed out a resemblance between the secretory process in the infected tissue with a cell-wall formation in the outer root-cap cells of maize (Mollenhauer *et al*, 1961). The stimulated production of endoplasmic reticulum and the development of encapsulation around the haustorium are analogous to secondary wall formation, as described by Porter and Machado (1960), who have shown with evidences that endoplasmic reticulum of plant cells is morphologically associated with plant-cell surfaces, where cell-wall formation is in progress or pending. The disintegration of encapsulation membrane (=host plasma membrane) coincided with the death of the host cell (Shaw and Manocha, 1965) and its significance as a protective layer is also reported by Hirata and Kojima (1962).

It could be assumed that in susceptible host cells, the delay in the development of encapsulation around the haustorium provides the fungus ample chance to nourish and to produce sporogenous structures when such supply is cut off, whereas in the resistant type, the early development of encapsulation deprives the fungus of host nutrition from the beginning. Whether or not the rapidity and efficiency with which this zone is formed have any connection with rust-resistance is not known with certainty, but great interest attaches to such a possibility.

Summary

The gross fine structure of haustoria of rust in susceptible and resistant host cells is similar insofar as mitochondria, endoplasmic reticulum and nuclei are concerned. Both types of host-cells after infection are stimulated to produce endoplasmic reticulum in the vicinity of a haustorium and to form encapsulation, a defensive barricade, but the resistant host-cell does this more quickly.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 1. Longitudinal section of haustorium in susceptible host cell, 6 days after inoculation, showing central nucleus (fn) with double membrane interrupted by pores (arrow), numerous mitochondria (fm) and endoplasmic reticulum (fer). Note the haustorial wall (fw) is closely adhering to the host plasma membrane (PM)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

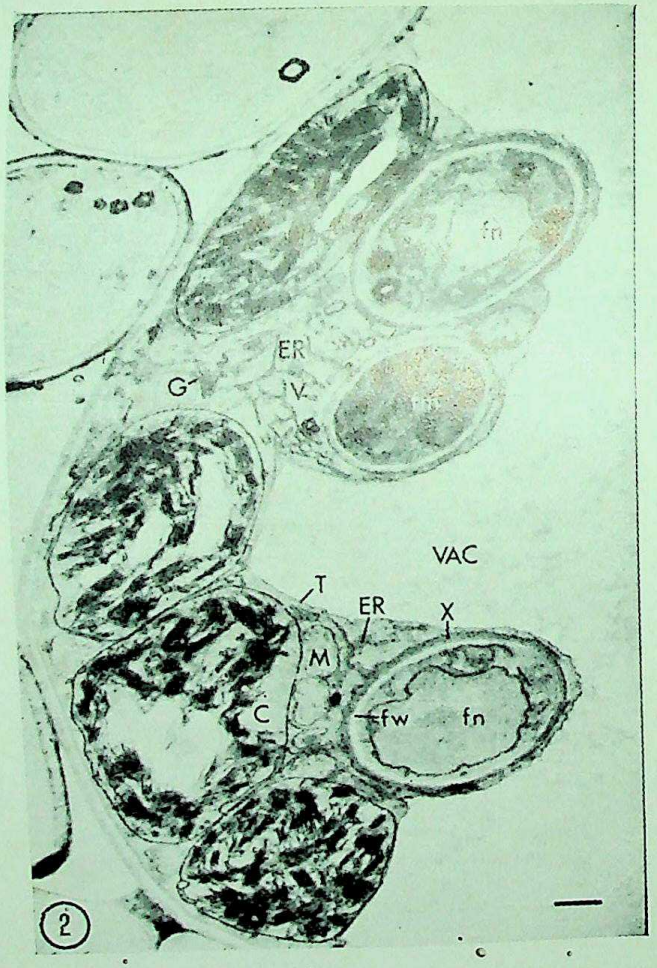


Fig. 2. Cross-section of haustoria in susceptible host cell 10 days after inoculation, showing long cisternae of host endoplasmic reticulum (ER) around them. The zone marked (X) appears between haustorial wall (fw) and host plasma membrane (PM)

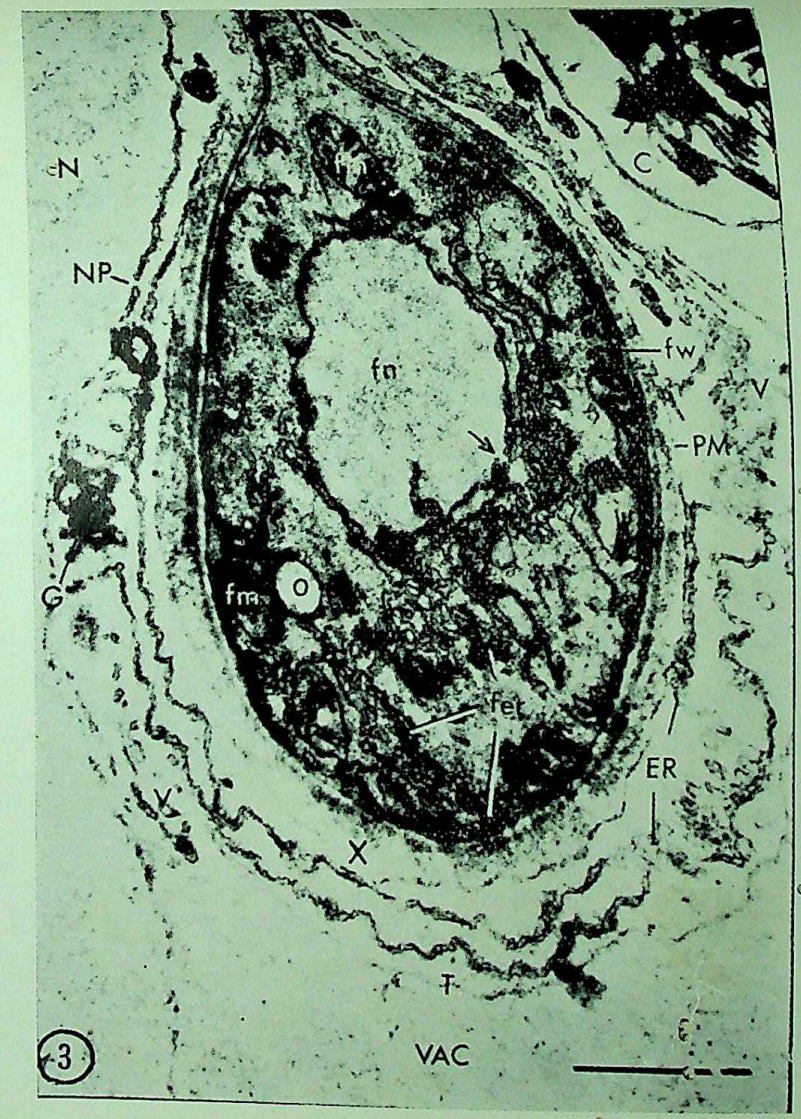


Fig. 3. Longitudinal section of haustorium in resistant host cell, 6 days after inoculation. Note the extensive system of host endoplasmic reticulum (ER) and development of encapsulation (X)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : The author is grateful to Professor M. Shaw, Head, Department of Biology, University of Saskatchewan, for providing necessary facilities.

Department of Biology
University of Saskatchewan
Saskatoon, Canada

REFERENCES

BERLIN, J. D. AND C. C. BOWEN, (1964) The host-parasite interface of *Albugo candida* on *Raphanus sativus*. *Amer. J. Botany* **51** : 445-452.

EHRLICH, H. G. AND M. A. EHRLICH, (1962) Fine structure of *Puccinia graminis var. tritici* in resistant and susceptible host varieties. *Amer. J. Botany* **49** : 665. Abstract.

EHRLICH, H. G. AND M. A. EHRLICH, (1963). Electron microscopy of host-parasite relationships in stem rust of wheat. *Amer. J. Botany* **50** : 123-130.

HAWKER, L. E. (1965) Fine structure of fungi as revealed by electron microscopy. *Biol. Rev.* **40** : 52-92.

HIRATA, K. AND M. KOJIMA, (1962) On the structure and the sac of the haustorium of some powdery mildews, with some consideration on the significance of the sac. *Trans. Mycol. Soc.* Japan **3** : 43-46.

MILLONIG, G. (1961) A modified procedure for lead staining of thin sections. *J. Biophys. Biochem. Cytol.* **11** : 736.

MOLLENHAUER, H. H., W. G. WHALEY, AND J. H. LEECH, (1961) A function of the Golgi apparatus in outer root-cap cells. *Jour. Ultrastruct. Res.* **5** : 193-200.

PEYTON, G. A. AND C. C. BOWEN, (1963) The host-parasite interface of *Peronospora manshurica* on *Glycine max*. *Amer. J. Botany* **50** : 787-797.

PORTER, K. R. AND R. D. MACHADO, (1960) The endoplasmic reticulum and the formation of plant cell wall. *Proc. European Regional Conf. of Electron Microscopy, Delft* **2** : 754-758.

SHAW, M. AND M. S. MANOCHA (1965) The physiology of host-parasite relations XV. Fine structure in rust-infected wheat leaves. *Can. J. Botany* (in press).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BIOCHEMICAL CHANGES IN THE BANANA FRUIT-COAT CAUSED BY GLOEOSPORIUM MUSARUM INFECTION

R. RAGHUNATHAN, A. MAHADEVAN, AND G. RANGASWAMI

(Accepted for publication December 10, 1965)

Varieties of banana are attacked by numerous fungal and bacterial pathogens. The commonly cultivated Monthan (*Musa sapidisiaca* Jacob) is susceptible to leaf-spot diseases, caused by *Cercospora musae* Zimm. and *Helminthosporium gibberosporium* Curzi. The leaves and fruits are also attacked by *Gloeosporium musarum* Cke. and Massee, resulting in the anthracnose disease.

Changes in protein, carbohydrate, and aromatic metabolism of the host are reported to occur during pathogenesis (Tomiyama, 1963). According to Rangaswami and Natarajan (1965), amino-acid metabolism of the banana plants is disturbed during pathogenesis. They showed that leucine, valine, proline, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine, lysine and crystine were present in the healthy Monthan leaves, while the leaves infected with *C. musae* contained low concentrations of valine, alanine, aspartic acid, arginine, tyrosine and lysine, The leaves infected with *H. gibberosporium* possessed small amounts of alanine, aspartic acid, arginine, and lysine. Lesser quantities of proline, alanine, glycine, serine, arginine, tyrosine and lysine were seen in the leaves infected with *Deightonella torulosa*

Wang (1960), studying the carbohydrate metabolism of the diseased bananas, reported that in the fruits infected with *G. musarum*, glucose, sucrose and other carbohydrates decreased. Barnell and Barnell (1945) suggested a causal relationship between the tannin content in banana fruits and *G. musarum* infection. Deese and Stahmann (1962) found that the pseudostem of a wilt-resistant variety contained more phenolic substances than the susceptible varieties. As soon as the fungus invaded the resistant plants, phenolic compounds accumulated, and in the presence of high oxidase enzymes, quinones formed, which inhibited the pathogenic enzymes.

In a previous communication (Raghunathan *et. al.*, 1965) it was indicated that some significant biochemical differences were found between the resistant Kattu Vazai and the susceptible Monthan banana varieties. It was suggested that an interaction of amino acid and phenol metabolisms of the hosts should receive attention. The present investigation reports the various biochemical alterations in the fruit peels of banana variety Monthan following *G. Musarum* infection.

MATERIAL AND METHODS : Banana species, *Musa sapidisiaca* Jacob variety Monthan, susceptible to leaf-spot and anthracnose diseases was used. Ripe fruits were surface-sterilized by soaking in 0.1 per cent

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

$HgCl_2$ solution for five minutes and thoroughly washed in sterile distilled water. They were inoculated with a ten-day-old culture of *G. musarum* and incubated in closed humidity chambers at room temperature (26-29°C). At intervals of 48, 120, 168 and 240 hrs., the fruits were removed from the chambers. Their peels were separated from the fruit-pulp, and analyzed for amino acids, carbohydrates and phenols.

Amino acids, carbohydrates including organic acids, and phenols were extracted from the fruit peels, as described earlier (Raghunathan *et al.*, 1965).

RESULTS : The healthy banana peel had nine amino acids just before inoculation. They were valine, alaine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine and asparagine. Immediately after inoculation with the fungus, their concentrations increased. However, after 120 hours., the quantity of all amino acids except that of glutamic acid decreased. With continued incubation, the amino-acid concentrations decreased whereas valine and glycine disappeared. Traces of amino acids were present after 240 hours. of inoculation and serine disappeared (Table 1). In the uninoculated fruits, the relative quantities of all amino acids remained almost the same throughout the period.

TABLE 1 : Changes in the amino-acid concentrations of Monthan fruit peel, as influenced by *Gloeosporium musarum* infections

Amino acid	Amino-acid content in mg/g on oven-dry basis								
	Time after inoculation (in hours)								
	0	48 cont.	48	120 cont.	120	168 cont.	168	240 cont.	240
Valine	1.78	2.72	1.72	1.78	1.47	1.78	0.00	1.76	0.00
Alanine	2.91	2.85	3.94	3.12	3.14	3.08	1.23	3.18	0.76
Threonine	0.79	1.19	1.66	1.28	0.89	1.21	0.57	1.22	0.48
Glycine	1.28	1.38	2.13	1.58	1.00	1.58	1.22	1.59	0.00
Serine	2.73	2.96	3.11	2.77	1.90	2.71	0.00	2.70	0.00
Glutamic acid	1.52	1.48	2.10	1.53	2.15	1.53	0.89	1.51	0.52
Aspartic acid	2.98	2.92	3 38	3.05	2.50	3.00	1.99	3.08	1.47
Arginine	1.75	1.82	2.55	1.99	2.44	1.95	1.40	1.94	1.05
Asparagine	2.54	2.27	3.45	2.43	3.14	2.41	1.39	2.40	0.93

Changes in sugar levels of fruit peels infected with *G. musarum* are shown in Table 2. Uninoculated peels contained fructose, glucose, and sucrose, whereas inoculated peels contained more sugars after 48 hours.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

After 120 hours, glucose and sucrose increased, while fructose descreased. With continued incubation, glucose increased, whereas fructose and sucrose decreased. At the end of 10 days, all the sugars decreased. Uninoculated fruit peels increased in sugar slightly.

TABLE 2 : Changes in the carbohydrates of Monthan fruit peel, as influenced by *Gloeosporium musarum* infection

Sugars	Glucose equivalents in mg/g on oven-dry basis								
	Time after inoculation (hours)								
	0	48 cont.	48	120 cont.	120	168 cont.	168	240 cont.	240
Fructose	0.144	0.310	0.310	0.31	0.281	0.29	0.21	0.30	0.17
Glucose	0.324	0.47	0·438	0.64	0.060	0.65	1.05	0.65	0.338
Sucrose	0.288	0.51	0.511	0.66	0.675	0.66	0.373	0.58	0.312

Changes in phenolic concentrations of the peels because of *G. musarum* infection are shown in Table 3. There was a gradual increase in phenols in the peel up to 120 hours, reaching a maximum at 168 hours and then decreasing to trace amounts. A parallel relationship was noticed in the uninoculated tissues although the net increase was less than that in the infected fruits.

TABLE 3 : Changes in total phenols in Monthan fruit peel, as influenced by *Gloeosporium musarum* infection

Time after inoculation	Total phenols in mg/g on oven-dry basis	
	Inoculated	Uninoculated
0 hr.	0.00	0.135
48 hrs.	0.193	0.184
120 hrs.	0.25	0.21
168 hrs.	0.48	0.21
240 hrs.	0.08	0.06

DISCUSSION : Metabolic changes in plant hosts are associated with disease development (Tomiyama, 1963 ; Uritani, 1963). Fruit peels infected with *G. musarum* increased in amino acids up to 120 hours and the increase probably acted as a protective mechanism against the fungus. Changes in amino acids, prior to infection were observed by Ponjar

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(1963) in potato virus infections on tobacco, by Rudolph (1963) In wheat infected with *Puccinia graminis tritici* and by Uritani (1963) in sweet-potato infected with *Ceratostomella ulmi*.

Immediately after infection, the amino-acid concentration decreased. Similar changes in amino acids following infection have been reported by numerous workers (Hrushovetz, 1954; Andal and Subba Rao, 1956; Chang *et al.*, 1959; Yamamoto and Honda, 1960, Burton and De-Zeeuw, 1961, Patel and Walker 1963, and McCombs and Winstead, 1964). Recently, Rangaswami and Natarajan (1965) showed a decline in amino-acid reserves following *C. musae*, *H. gibberosporium* and *D. torulosa* infections of banana leaves. From this study, it appears that immediately after the infection, free amino acids are transported to the site of infection for the development of the pathogen.

The carbohydrate metabolism of the fruits also followed the same pattern as that of amino acids. Fructose, glucose and sucrose levels increased in the tissues infected with *G. musarum* up to 120 hours after inoculation. Immediately after infection, the amount of sucrose and fructose decreased. A Similar decrease in free sugars is known in pathogenesis (Wolf and Wolf, 1955; Herzmann, 1958; Krog *et al.*, 1961; Ghosh *et al.*, 1964; McCombs and Winstead, 1964). The decline in the carbohydrate reserves might be attributed to the utilization of these compounds by the pathogen. Post-infectional changes in the phenolic compounds of host plants is very characteristic in many plant diseases (Kiraly and Farkas, 1962; Kiraly, 1962 ; Mahadevan *et al.*, 1965) and banana peels exhibited the same trend.

Activation of amino acids, carbohydrates and aromatic compounds was characteristic in the banana fruit peels infected with *G. musarum* and with the onset of symptoms, these concentrations decreased. Amino acids and simple sugars were transported to the site of infection for the nutrition of the parasite, which in turn increased its invasiveness of the host's defence mechanism.

SUMMARY

The biochemical changes in banana fruit-coat caused by *Gloeosporium musarum* Cke. and Massee, the anthracnose fungus, were investigated. The fruits of the variety Monthan (*Musa sapidisiaca* Jacob) were inoculated with *G. musarum* and incubated at room temperature (28-30° C) for 2, 5, 7 and 10 days, during which intervals the changes in the amino-acid, carbohydrate and phenol contents of the fruit-coat were examined, following the standard procedures. The healthy banana fruit peels contained nine amino acids, *viz.* valine, alaine, threonine, glycine, serine, glutamic acid, aspartic acid, arginine and asparagine. The concentrations of these amino acids increased soon after inoculation with the fungus, but declined after about 120 hours, and owing to the continued incubation valine and glycine disappeared. The healthy fruit peels contained fructose, glucose and sucrose and their concentration increased with inoculation. Because of fungal infection their quantity increased after 48 hours, but with continued incubation the concentration

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

of glucose increased, while that of fructose and sucrose decreased. The phenol content of the fruit peels increased soon after fungal infection but after about 168 hours it decreased, and was finally reduced to traces. In the uninoculated fruit peels, the reductions in the phenol concentrations, though parallel, were less marked.

Annamalai University
Annamalainagar
Madras State

References

ANDAL, R. AND N. S. SUBBA RAO (1956) Amino acid changes in the healthy and foot rot infected rice. *J. Indian Bot. Soc.*, **35** : 414-16.

BARNELL, H. R. AND E. BARNELL (1945) Studies in tropical fruits. XVI. The distribution of tannins within the banana and the changes in their conditions during ripening. *Ann. Bot. N. S.* **9** : 79-99.

BURTON, CLYDE, L. AND DEZEEUW, D. J. (1961) Free amino acid constituents of healthy and scab-infected foliage. *Phytopathology*, **51** : 776-777.

CNANG, Y. O., C. Z. MCANELLY AND J. R. VAUGHN (1959) A comparison of the amino acid content of beans produced from healthy and Fusarium root rot infected plants. *Plant Dis. Rept.* **43** : 437-38.

DEESE, D. C. AND M. A. STAHMANN (1962) Pectic enzymes and cellulase formation by *Fusarium oxysporum* f. *cubense* on stem tissue from resistant and susceptible banana. *Phytopathology* **52** : 247-255.

GHOSH, A. K., R. N. TANDON, K. S. BILGRAMI AND M. P. SRIVASTAVA (1964) Post-infection changes in sugar contents of some fruits. *Phytopathol. Z.* **50** : 283-288.

HORZMANN, H. (1958) Biochemical studies on the metabolism of healthy and anthracnose diseased beans. I. Studies on carbohydrates, nitrogen compounds, carboxylic acids and some oxidative enzymes. *Phytopathol. Z.* **33** : 349-370.

HRUSHOVETZ, S. B. (1954) The effect of infection by *Helminthosporium sativum* on the amino acid content of wheat roots. *Can J. Botany* **32** : 571-575.

KIRLAY, Z. (1962) Phenol content in rust-infected and nitrogen-fertilized wheat leaves. *Phytopathology* **52** : 738 (abst.)

——— AND G. L. FARKAS (1962) Relation between phenol metabolism and stem rust resistance in wheat. *Phtopathology* **52** : 657-663.

KROG, N. E., D. LE TOURNEAU AND H. HART, (1961) The sugar content of wheat leaves infected with stem rust. *Phytopathology* **51** : 75-77.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

MAHADEVAN, A., J. KUC' AND E. B. WILLIAMS (1965) Biochemistry of resistance in cucumber against *Cladosporium cucuminum*. I. Presence of a pectinose inhibitor in resistant plants. *Phytopathology* **55** : 1000-1003.

MCCOMBS, C. L. AND N. N. WINSTEAD (1964) Changes in sugars and amino acids of cucumber fruits infected with *Pythium aphanidermatum*. *Phytopathology* **54** : 233-334.

PATEL, P. AND J. C. WALKER (1963) Free amino acids and amide content of tobacco and oats infected by wildfire and halo blight bacteria. *Phytopathology* **53** : 885 (abst).

PONJAR, E. (1963) Changes occurring in the free amino acid composition of healthy and virus infected tobacco leaves. *Acta Biol. Cracor. Ser. Bot.* **5** : 171-179 (in *Rev. Appl. Mycol.*, **43** : 211, 1964).

RAGUNATHAN, R., G. RANGASWAMI, AND A. MAHADEVAN (1965) A biochemical study of resistant and susceptible banana varieties to fungal diseases. *Ind. Phytopath* (in press).

RANGASWAMI, G. AND S. NATARAJAN (1965) Studies on some chemical components of banana leaves and fruits, as influenced by fungal infections. *Phytopathology* (in press).

RUDOLPH, K. (1963) Further biochemical studies on host-parasite relationship, as exemplified by *Puccinia graminis tritici*. I. Effect of infection on acid substance exchange. *Phytopathol. Z.* **46** : 276-290.

TOMIYAMA, K. (1963) Physiology and biochemistry of disease resistance of plants. *Ann. Rev. Phytopath.* **1** : 295-324.

URITANI, I. (1963) The biochemical basis of disease resistance induced by infection. In Perspectives of Biochemical Plant Pathology, edited by Rich, S. *The Conn. Ag. Exp. Station, New Haven* pp. 4-19.

WANG, M. C. (1960) Physiological studies on *Gloeosporium musarum*, the causal organism of banana anthracnose. I. Changes in the carbohydrate composition of banana pulp, with special reference to the adaptive secretion of amylase. *Bot. Bull. Acad. Sci. N. S.* **1** : 59-75.

WOLF, F. A. AND F. T. WOLF (1955) The chemical composition of leaves of diseased tobacco ring spot, bacterial wilt and fusarial wilt. *Phytopathology* **45** : 506-508.

YAMAMOTO, M. AND H. HONDA (1960) On the amino acid content in potato leaves at different stages of growth and potato tubers infected by *Phytophthora infectants*. *Ann. Phytopath. Soc. Japan* **25** : 209-213.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EFFECT OF CERTAIN VITAMINS ON SCLEROTIUM CEPIVORUM BERK.

L. K. JOSHI AND M. P. HAWARE

(Accepted for publication February 28, 1966)

Sclerotium cepivorum Berk. causes a serious rot of onion bulbs and is widely distributed throughout the onion-growing areas in India and the world. Some cultural studies were carried out by Asthana (1947), but no work seems to have been done on the vitamin requirement of the fungus. Studies in this respect are reported in this paper.

MATERIAL AND METHODS : *Sclerotium cepivorum* Berk. was isolated from the rotted onion bulbs obtained from the Botanical Garden of the College of Agriculture, Gwalior, and the pathogenicity of the fungus was proved experimentally. The fungus was subcultured once a fortnight on potato-dextrose-agar.

Richard's solution was used for the study of the vitamin requirement of the fungus. The medium was adjusted to pH 6.0 with a Beckman pH meter. To determine the vitamin deficiency of the fungus, the following vitamin concentrations/litre were used : thiamine, pyridoxine, nicotinic acid, pantothenic and p-aminobenzoic acid, 200 μg/l each; biotin and riboflavin 100 μg/l each; and Inositol 40 μg/l. An aliquot of 20 ml of the medium per 100 ml Pyrex flask was used to which required quantity of each vitamin solutions was added. These were sterilized at 15 lb pressure for 20 minutes. All glassware used were of Pyrex-make. Water redistilled in Pyrex-glass and reagent-grade chemicals (Analar) were used throughout the investigation. Different nitrogen and carbon sources were substituted for sucrose and KNO_3 of the basal medium by providing equivalent amounts of carbon and nitrogen respectively. The inoculum was prepared from a 7-day-old culture, very sparsely growing in Richard's solution. Inoculation was done by introducing a standard loopful of mycelial suspension in each flask. The inoculated flasks were incubated for 12 days at 25°C. The dry weight of the mycelium was determined and thereafter the mean of the growth in four flasks was expressed in mg.

EXPERIMENTAL : *Determination of Vitamin Deficiency* : The method used by Burkholder (1943) for detecting vitamin deficiencies of yeasts was followed. The deficiency was detected by the inability of the fungus to grow in a medium which was complete, except for one vitamin. Failure to grow in a medium indicated a deficiency for the vitamin omitted.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Effect of certain vitamins on the growth of *Sclerotium cepivorum*

Vitamin added to the basal medium	Average dry weight in mg.
No vitamin	131
None omitted	421
Thiamine omitted	136
Biotin omitted	342
Pyridoxine omitted	398
Inositol omitted	336
Pantothenic acid omitted	300
Nicotinic acid omitted	359
Riboflavin omitted	402
p-aminobenzoic acid omitted	320

The data presented in Table 1 indicate that *S. cepivorum* shows poor growth when the basal medium is devoid of vitamins or thiamine. The absence of other vitamins tested had no appreciable effect on the growth of the fungus.

TABLE 2 : Effect of different concentrations of thiamine on the growth of *S. cepivorum*

Thiamine concentration (μg/l)	0	1	5	10	25	50	100	200	400	800
Dry wt. (mg.)	164	222	215	255	381	370	273	198	202	199

The data in Table 2 indicate that *S. cepivorum* shows increase in growth up to 25 μg/l of thiamine. However, further increase in thiamine concentration reduces the growth of the fungus.

TABLE 3 : Effect of pH on the thiamine requirement of *S. cepivorum*

Thiamine concentration μg/l	Average dry weight of mycelium (mg.)				
	pH 4	pH 5	pH 6	pH 7	pH 8
0	184	191	201	95	88
1	188	200	314	152	128
10	220	396	409	175	133
100	403	336	397	169	96
1000	385	307	301	178	110

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The fungus grows best between pH 5.0 and 6.0. The growth optima of the organism were not changed with the addition of thiamine except at higher concentrations (100 and 1000 μg/l), where the best growth was obtained at pH 4.0.

TABLE 4 : Growth of *S. cepivorum* in the presence of different vitamins and nitorgen sources, when the carbon source is sucrose or glucose

Vitamin added to the basal nedium	Average mycelial dry weight (mg.)							
	Potassium nitrate		Asparagine		Ammonium sulphate		Urea	
	Sucrose	Glucose	Sucrose	Glucose	Sucrose	Glucose	Sucrose	Glucose
No vitamin	119	196	194	180	70	76	66	71
None omitted	246	490	328	590	237	571	225	225
Thiamine omitted	123	181	187	176	72	78	65	78
Biotin omitted	244	535	328	594	255	561	234	244
Pyridoxin omitted	234	559	331	587	257	582	229	301
Inositol omitted	244	503	333	552	232	571	215	281
Pantothenic acid omitted	236	468	301	538	233	534	225	302
Nicotinic acid omitted	227	511	300	565	228	565	230	242
Riboflavin omitted	231	498	275	587	255	555	236	305
p-aminobenzoic acid omitted	223	454	285	551	224	566	215	298

The data presented in Table 4 indicate that the growth of *S. cepivorum* was poor when all vitamins or thiamine were omitted from the medium containing sucrose or glucose as carbon sources and potassium-nitrate, asparagine, ammonium sulphate or urea as sources of nitrogen. The fungus shows increase in growth in all other treatments where vitamins other than thiamine were omitted. The fungus showed better response when asparagine was the source of nitrogen.

From the above observations it is clear that thiamine deficiency of the fungus is not modified, when carbon or nitrogen sources are altered in the basal medium.

DISCUSSION : Various workers have demonstrated the vitamin deficiency of filamentous fungi. *S. cepivorum* is heterotrophic with respect to thiamine as it shows poor growth in the absence of thiamine in Richard's solution.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The fungus responds to 1 μg/l of thiamine in the medium. John reported (as stated by Watkins, 1961) that at the lowest level of 0.01 ppm thiamine, abundant growth of *S. rolfsii* was obtained, which was comparable with that obtained at higher concentrations used. *S. cepivorum* showed optimum growth response at 25 μg/l of thiamine. Lilly and Barnett (1950) stated that for most fungi 100 μg/l of thiamine in the medium is the near optimum for growth and sporulation. Suryanaraynan (1959) obtained maximum growth of *S. oryzae* with 1.0 μg and 10.0 μg of thiamine per flask (25 ml medium). This dose looks to be high when compared with the dose for the maximum growth obtained in the present experiment.

In the present investigation it has been found that low cencentrations of thiamine stimulate growth of the fungus at pH 5.0-6.0, which is also optimum for the normal growth of the fungus. The pH of the medium does not appear to alter the thiamine heterotrophy of *S. cepivorum*. Similar results have been obtained by Suryanarayanan (1959) while working with *Piricularia sp.* Turel (1952) reported inhibitory action of thiamine on *Fusarium solani* in its later stage of growth. In hibitory effects of vitamins, when used in excess quantities, are apparently common (Lilly and Barnett, 1949). Thiamine is more commonly reported as a growth-depressor than other vitamins.

The thiamine deficiency of the fungus was not modified in any combination of the carbon or nitrogen sources tried. Thus, thiamine deficiency of *S. cepivorum* appears to be complete and not conditioned. Suryanarayanan (1959) also found similar results with *S. oryzae*.

Summary and Conclusions

In the present investigation it has been found that *S. cepivorum* is deficient only for thiamine. *S. cepivorum* shows response to 1 μg/l of thiamine in Richard's solution. Optimum growth response is obtained at 25 μg/l thiamine. Higher doses of thiamine do not exercise inhibitory effect on the growth when compared with that in the case of the control. Thiamine deficiency of the fungus was tested at various pH levels of the medium. The optimum dose of thiamine required by the fungus varies with the pH of the medium. Higher doses of thiamine (100 and 1000 μg/l) decrease the growth of the fungus at all pH levels tested. The thiamine deficiency of *S. cepivorum* was tested with two carbon sources in combination with four nitrogen sources. It has been found that the thiamine deficiency is not modified in any of the modifications of the medium by using different carbon and nitrogen sources.

Acknowledgement : Our grateful thanks are due to the Principal, College of Agriculture, Gwalior (Gwalior Campus of the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur) for providing facilities in carrying out the work.

Department of Plant Pathology
College of Agriculture
Gwalior M. P.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

ABEYGUNAWARDENA, D. V.W. AND R. K. S. WOOD (1957) Factors affecting the germination of sclerotia and mycelial growth of *S. rolfsii*. *Trans. Brit. mycol. Soc.*, **40** : 221-23.

ASTHANA, R. P. (1947) Studies on sclerotium forming fungi-I, *S. cepiorum* Berk and *S. tuliparum* Klenbohn. Part I. Cultural Studies. *Proc. Indian Acad. Sci.*, **26B** : 93-107.

BARNETT, H. L. AND V. G. LILLY (1948) Effect of vitamins on growth of *Sclerotium camelliae*. *Amer. J. Bot.*, **36** : 297-211.

BURKHOLDER, P. R. (1943) Vitamin deficiencies in yeasts. *Amer. J. Bot.*, **30** : 206-211.

COCHRANE, V. W. (1958) Physiology of fungi. John Wiley and Sons. Inc. New York.

FRIES, N. (1948) The nutrition of fungi from the aspect of growth factor requirements. *Trans. Brit. mycol. Soc.*, **30** : 118-134.

HAWKER, LILLIAN E. (1950) Physiology of fungi. McGraw Hill Book Company. Inc., New York, London.

MISHRA, A. P. AND M. MAHMOOD (1961) Effect of vitamins and hormones on growth and sporulation of *Colletotrichum capsici*. *Ind. Phytopath.* **14** : 20-22.

ROBINS, W. J. AND F. KAVANAGH (1938) Vitamin deficiencies of filamentous fungi. *Bot. Rev.*, **8** : 411-471.

SURYANARAYANAN, S. (1958) Growth factor requirements of *Piricularia spp.* and *Sclerotium oryzae*. *Proc. Ind. Acad. Sci.* **48B** : 154-188.

————————(1958) Thamine, its intermediates and growth of *Piricularia spp.* and *S. oryzae*. *Phytopath. Z.* **33** : 341-348.

TAUBENHAUS, J. J. (1919) Recent studies on *S. rolfsii* Sacc. *J. agric. Res.* **18** : 127-138.

WATKINS, G. W. (1961) Physiology of *S. rolfsii* with emphasis on parasitism. *Phtopathology* **51** : 110-113.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON THE PERENNATION AND SURVIVAL OF SESAMUM PHYTOPHTHORA*

S. P. SEHGAL AND N. PRASAD

(Accepted for publication January 10, 1966)

INTRODUCTION : The ability of a pathogen to survive depends on the methods of its reproduction and ways of its overcoming unfavourable periods. In nature, pathogenic organisms may survive in various forms on some alternate host or on some collateral host, in soil or in seed in the absence of host. The isolate of *Phytophthora* attacking sesamum (*P. parasitica* var. *sesami* Prasad) has been found to infect only sesamum species and it was also observed that it did not form oospores either in culture or on the host (Kale and Prasad, 1957 ; Gemawat, 1956; Sehgal, 1963). Due to these facts, the problem of its survival during the absence of its crop becomes rather interesting.

Soils and seeds have been considered as potential sources of infection in many cases. Nolla (1928) reported that the main source of infection of the black shank disease of tobacco caused by *P. parasitica* var. *nicotianae* is the tobacco plant debris left over from the preceeding crop and according to him the fungus may live in the soil as a saprophyte on contaminated manure for five or six years. The root rot of tomatoes caused by *P. parasitica* has been reported both in the nursery and the field where the fungus survives in soil for one year or more (Reddick, 1920 ; Richardson, 1941 ; Fulton and Fulton, 1951). Tsao (1958) isolated *P. citrophthora* and *P. parasitica* from soil and estimated potential disease index. Many species of *Phytophthora* causing various diseases have been isolated by many workers from soil by using baits, etc. (Smith, 1955 ; Zan, 1956 ; Klotz and DeWolfe, 1958 ; Newhook, 1959).

Disease-free seed plays an important role in the production of healthy crops. Orton (1926) listed more than 200 parasitic diseases known to be carried by seeds. Machacek (1929) reported that the interior of onion bulbs may be infected by *Aspergillus niger* without any external evidence of the presence of the fungus and may act as a potential source of further infection. In the case of bean anthracnose which is caused by *Colletotrichum lindemuthianum*, the fungus survives from one season to the next in the form of dormant mycelium in the testa or within the cells of the cotyledons (Barrus, 1921). Cook (1935) considered that the internal infection of the seed of spinach might take place in severely diseased fields and such seed gives severely mildewed crop in the next season. In wheat and barley the embryo is penetrated by the loose smut fungus, and passes a dormant period in it. Mycelium of *P. infestans* also remains dormant in the potato tubers and gives rise to blighted sprouts in the next season. In the following studies, detailed investigations have been made on the perennation and survival of the *Phytophthora* attacking sesamum.

*Part of thesis submitted by the senior author and approved for the Ph. D. degree by the Rajasthan University.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

EXPERIMENTAL : *Persistence and survival of sesamum Phytophthora in soil.*

During investigations on the blight disease of sesamum which is caused by *P. parasitica* var. *sesami*, it has been observed that the disease occurs in an epiphytotic form if the same field is used again and again. A heavy incidence of the disease was noticed in a field where the sesamum crop had been taken successively for two years (Sehgal, 1963).

To study the role of soil in the perennation of the disease, experiments were conducted using soil in which heavy incidence of the disease had been noticed in previous years. In July 1960, sesamum plants were raised in 9″ pots and inoculated when they were six weeks old with a virulent isolate No. 2 by adding the fungus culture to the soil and then covering it with a little of ordinary soil. Such plants were watered heavily. All plants were killed within 15 days after inoculation. After the sesamum season, when the soil had dried completely, stubs of infected plants were removed from the pots. Such pots along with the soil and plant debris were left in the open for about nine months (October to June) under natural conditions. Temperatures during the period varied from 4° to 44° C. A few light shower of rain also occurred during the period. Side by side, the control pots were also kept under identical conditions except that no inoculations were made in them.

On the 27th. June, 1961, soil from such pots was removed and pulverised and again filled in the pots. In each pot, 20 surface-sterilized sesamum seeds were sown, in control pots also similar sowings were made. In the beginning, germination was normal in both the cases. But on the 11th day a few plants showed symptoms in pots with infested soil and within three days all plants collapsed and died. The control plants remained normal and on maturity formed healthy capsules. A pure culture of *Phytophthora* was isolated from the blighted plants ; this was identical in all respects with the sesamum *Phytophthora*. The experiment was repeated again in the year 1962 with similar results.

Direct isolations of *Phytophthora* were also successfully made from the infested soil by using baits. Ripe tomato fruits were kept in pots containing one-year-old diseased soil, and watered heavily. Infection on fruits took place within three days. The isolations made from fruits were identical in all morphological details with the *Phytophthora* isolated from sesamum.

(b) *Probable role of seed as a source of initial infection* :

From symptomatology of the blighted sesamum plants it was observed that the disease was not confined to the stem or the leaves but the capsules were also attacked. In such capsules seeds are poorly formed. Severely infected seeds always failed to germinate. Isolations made from fresh capsules and seeds yielded pure cultures of *Phytophthora*. On freshly infected capsules a fine growth of fungus appears ; when such capsules are cut open, the growth of the fungus can be seen inside and also on young developing seeds. When sections of such capsules along with the seeds are cut, the mycelium can be seen penetrating the seeds.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The mycelium penetrates the capsules and then through seed-coat it reaches the nucellus and the endosperm and thence enters the embryo. Within the maturing seeds the mycelium swells considerably and becomes thickened. It is possible that the pathogen passes the unfavourable period in its life history, a resting or dormant form in the seeds.

Attempts were made regularly to isolate pure cultures of *Phytophthora* from naturally infected stored seeds. A pure culture of *Phytophthora* has been obtained from three-month-old seeds ; but later on it was not possible to obtain a pure culture from still older seeds. It is also interesting to note that isolations of *Phytophthora* were not possible from older infections on stem, leaf or capsule.

DISCUSSION : All pathogenic diseases are transmissible, otherwise the pathogens would perish in due course. For successful infection, presence of a pathogen is essential in some form, in addition to the favourable environmental conditions. Sesamum blight has been observed successively for years if the same field is used again and again. Soil and seed play an important role in the transmission and recurrence of the disease. The present studies have shown that the blight fungus, i. e. *P. parasitica* var. *sesami* can survive in the soil for about a year in the absence of any crop. McRae (1934) reported the thermal death-point of *P. parasitica* to be 48°C. Gemawat (1956) reported thermal death-point of *P. parasitica* var. *sesami* to be 48°C. Sehgal (1963) observed that young cultures of sesamum *Phytophthora*, *P. parasitica* var. *sesami* which had no chlamydospores could withstand 50°C. for 10 minutes, and old cultures in which chlamydospores were present could withstand 52°C. for 10 minutes. These high thermal death-points help the pathogen to survive during summers in soil where temperature never rises above 50°C. The fungus survives in soil either in the from of dormant mycelium and/or in the form of chlamydospores and not in the form of oospores, as in this case they are never formed. From the sick soil the fungus can also be isolated by placing tomato baits. When healthy seeds are sown in such soil, the disease appears and causes serious damage.

Seed can be another source of initial infection and might be playing an important role in the recurrence and spread of the disease. In the seeds the mycelium has been located in the embryo and successful isolations have been made from four-month old seeds. But from still older seeds pure culture of *Phytophthora* could not be made. In sections and teased seeds, coenocytic mycelium has always been observed. It is possible that the mycelium might be living inside the seeds in a dormant form and in some cases might be the initial source of infection. This view is further strengthened as disease has been observed in many cases in fields which were never put under plough before the sowing of sesamum crop in the field.

SUMMARY

The blight of sesamum is an important disease caused by *Phytophthora parasitica* var. *sesami*. Serious damage has been observed in the fields when the crop was sown successively for years. Experiments

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

have shown that the fungus can survive in the soil for one year and under favourable conditions causes infection of sesamum plants sown in such soil. The fungus survives in the soil in the form of mycelium or chlamydospores as it does not form oospores. Direct isolations of the fungus from infested soil are also possible by using ripe tomato fruits as baits.

It is also considered that seed may play some role in the initiation and spread of disease in new localities. In such cases, mycelium of the fungus lives in the embryo in a dormant condition.

ACKNOWLEDGEMENTS : We are grateful to the Director of Agriculture, Rajasthan, and to Dr. R. L. Mathur, Plant Pathologist, Rajasthan, for facilities provided during the course of the studies.

Plant Pathology Section
Sub-Station, Kota
Rajasthan

REFERENCES

BARRUS, M. F. (1921) Bean Anthracnose. *Cornell Agri. Stn. Mem.* **42** : 101.

COOK, H. T. (1935) Occurrence of oospores of *Peronospora effusa* with commercial spinach seed. *Phytopathology* 25 : 11-12 (Absts).

FULTON, J. P. AND D. NEIL FULTON (1951) *Phytophthora* root rot of tomatoes. *Phytopathology* **41** : 99-101.

GEMAWAT, P. D. (1956) Further studies on *Phytophthora* blight of sesamum. M. Sc. thesis, Gujarat University.

KALE, G. B. AND N. PRASAD (1957) *Phytophthora* blight of sesamum. *Indian Phytopath.* **10** : 38-47.

KLOTZ, L. J. AND T. A. DEWOLFE, T. A. (1958) Techniques for isolating *Phytophthora* spp. which attack Citrus. *Plant Dis. Rept.* 42 : 675-676.

MACHACEK, J. E. (1929) The Black mould of onion caused by *Aspergillus niger* V. Tiegh. *Phytopathology* **19** : 733-739.

MCRAE, W (1934) Foot rot disease of *Piper betel* L. in Bengal. *Indian J. agric. Sci.* **4** : 585-617.

NEWHOOK, F. J. (1959) The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers I. Symptoms and epidemiology in shelter belts. *N. Z. J. Agri. Res.* **2** : 808-842.

NOLLA, J. A. B. (1928) The black shank of tobacco in Porto Rico. *J. Dept. Agr. Porto Rico* **7** : 185-215.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ORTON C. R. (1926) Seeds as carriers of disease. *Journ. New York Bot. Gard.* **27** : 54-63.

REDDICK, D. (1920) A fourth *Phytophthora* disease of tomato. *Phytopathology* **10** : 528-534.

RICHARDSON, L. T. (1941) A *Phytophthora* disease of tomato new to Ontario. *Can. J. Res.* (c) **19** : 446-483.

SEHGAL, S. P. (1963) Studies on the *Phytophthora* blight of sesamum in Rajasthan. Ph. D. thesis, Rajasthan University.

SMITH, H. C. (1955) Collar rot and crown rot of Apple trees. *Orchard N. Z.* **28** : 16-21.

TSAO, P. H. (1958) Serial dilution method for estimating disease potentials of *Phytophthora* in soil. *Phytopathology* **48** : 398-399.

ZAN, K. (1956) Persistence and movement of *Phytophthora infestans* in soil. *Trans. Brit. mycol. Soc.* **39** : 385. (Abstr).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

VIABILITY OF FUNGICIDE-TREATED WHEAT AND BARLEY SEEDS IN STORAGE

J. S. GREWAL AND S. KAPOOR

(Accepted for publication February 26, 1966)

Cereal seeds are commonly treated with fungicides for protection against seed and soil-borne fungi. In the Punjab large quantities of wheat seeds are treated with organomercurial fungicides by the plant protection staff for sowings within or outside the State. In some years, bulk of the treated seeds cannot be used for immediate sowings owing to unavoidable reasons.

Very little work has been done on the viability of fungicide-treated seeds in storage, except by Brett and Weston[1]. Lack of reliable information on this aspect has resulted in the disposal of treated seeds at cheaper rates and has indirectly discouraged bulk seed-treatment. Keeping in view the practical importance of the problem, experiments were conducted to test the viability of fungicide-treated wheat and barley seeds stored in three types of containers for a period of three years.

MATERIAL AND METHODS : Seeds of wheat (variety C 518) and barley (NP 13) were obtained from the Agronomy Division, Indian Agricultural Research Institute, New Delhi. Each was divided into ten lots. Nine lots were treated with fungicides and tenth one was left untreated to serve as control. Seeds were treated with Ceresan (phenylmercury acetate), copper carbonate, Fernasan [bis (dimethylthiocarbamoyl) disulphide], Agrosan GN (tolylmercury acetate), Tillex (ethylmercury chloride), Agrosan 5 W (ethylmercury chloride ; phenylmercury acetate), Fusariol (ethylmercury cyanide), Phygon (2-3-dichloro-1, 4-napthoquinone) and Flit 406 [n-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide].

Weighed amounts of wheat and barley seeds and weighed amounts of fungicides were added in conical flasks separately. Seeds and fungicides in flasks were shaken on an electric shaker for twenty minutes to ensure uniform coating of the fungicides on the seeds. Treated wheat and barley seeds as well as untreated controls were divided into equal lots and stored in three types of containers, e.g. air-tight bottles, alkathene-lined gunny bags and ordinary gunny bags. Alkathene used was of 700-guage thickness. Seeds in these containers were stored in the laboratory at room temperature (15° C to 40° C) where relative humidity ranged from 30 to 71 per cent. The viability (germination) of treated and untreated seeds was tested in the laboratory on moist blotting paper in petri plates at 25°C and in soil under glasshouse conditions. The germination of seeds was tested one day after the treatment and subsequently at the end of one, two and

1. Brett, C. C. and W.A.R. Dillon Weston (1941). *J. agric. Sci.* 31 : 500-517.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

three years of storage. One hundred seeds in four replications were used for recording their viability. The germination was recorded 6 days after sowing in the laboratory and 10 days after sowing in the case of glasshouse tests. The percentage germination data of wheat and barley in different treatments stored in three types of containers for a period of three years in the laboratory as well as germination data obtained from tests in the glasshouse were analysed statistically after suitable angular transformation of the data for each crop separately. The transformation formula used was $\sin^{-1}\sqrt{p}$ where p is the percentage germination.

RESULTS : The results in transformed values of percentage germination are summarized in Tables 1 and 2. Analysis of the data indicated that in all cases germination was significantly higher in the laboratory than that in pots in the glasshouse.

Wheat : It is evident from the data in Table 1 that the viability of seeds treated with ceresan, copper carbonate, Fernasan, Agrosan GN, Tillex and Agrosan 5W is significantly higher while that of seeds treated with Fusariol, Flit 406 and Phygon is significantly lower than that in the untreated control. The highest viability was recorded in ceresan-treated seeds. Seeds stored in alkathene-lined gunny bags and air-tight bottles retained significantly higher viability than those stored in ordinary gunny bags.

Treatment × Containers : Viability of seeds treated with Fusariol, Phygon and Flit 406 was significantly higher in alakathene-lined bags and significantly lower in air-tight bottles than that in ordinary gunny bags. The viability was significantly higher when seeds treated with copper carbonate, Tillex and Agrosan 5W as well as the untreated control were stored in air-tight bottles than that in the other two containers.

Treatment × Years : There was no significant reduction in the viability of seeds treated with Ceresan, Fernasan, Agrosan 5W, Fusariol Phygon, Flit 406 and in the untreated control at the end of one year's storage. Viability was significantly reduced after two and three years of storage in many treatments. Highest germination was recorded in Ceresan-treated seeds at the end of 2 and 3 years' storage, while it was significantly lower in the case of seeds treated with Fusariol, Phygon and Flit 406.

Barley : The results regarding the viability of treated barley seeds stored in three different types of containers are summarized in Table 2.

It is evident from the data in Table 2 that the viability is significantly higher in Fernasan and Agrosan 5W treated seeds and significantly lower in the case of Flit 406 treatment than that in untreated control. Viability of barley seeds was significantly more in-air-tight bottles and alkathene-lined bags than that in ordinary gunny bags.

Treatment × Containers : The viability of seeds treated with Agrosan 5W, Agrosan GN, Copper carbonate, Phygon and that in the untreated control was significantly higher in air-tight bottles than that of seeds stored in ordinary gunny bags.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 1 : Viability of wheat seed treated with different fungicides stored in three types of containers for a period of three years (Figures in transformed values)

Fungicides	Dosage (per cent)	Types of containers			Period of storage in years				Mean
		Air-tight bottles	Alkathene-lined bags	Gunny bags	0	1	2	3	
Ceresan	0.2	72.9	70.7	58.2	78.6	73.5	68.9	48.2	67.3
Copper carbonate	0.3	73.5	65.1	55.7	80.2	71.0	62.9	45.1	64.8
Fernasan	0.3	71.5	68.2	53.2	75.9	71.6	62.4	47.3	64.3
Agrosan GN	0.3	68.4	68.3	54.9	79.3	71.1	61.3	43.9	63.9
Tillex	0.2	72.9	65.5	53.2	76.8	71.0	63.7	44.1	63.9
Agrosan 5W	0.07	71.4	64.9	54.3	73.7	72.4	67.0	41.0	63.5
Fusariol	0.2	43.3	58.5	50.2	70.7	65.5	43.6	22.9	50.7
Phygon	0.15	39.1	58.5	47.0	70.7	66.7	38.5	16.8	48.2
Flit 406	0.2	36.8	60.1	47.2	68.9	64.3	38.5	20.4	48.0
Control	—	67.2	62.2	52.4	70.2	66.9	59.4	45.9	60.6
Mean		61.7	64.2	52.6	74.5	69.4	56.6	37.5	

C. D. at 5 per cent level for :—

Treatments	=	2.7
Containers	=	1.5
Years	=	1.7
Treatment × Containers	=	4.7
Treatment × Years	=	5.5

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

TABLE 2 : Viability of barley seed treated with different fungicides and stored in three types of containers for a period of three years (Figures in transformed values)

Fungicides	Dosage (per-cent)	Types of containers			Period of storage in years				Mean
		Air-tight bottles	Alkathene-lined bags	Gunny bags	0	1	2	3	
Fernasan	0.30	78.2	78.0	73.7	80.2	78.5	75.0	72.7	76.6
Agrosan 5 W	0.07	79.2	78.4	71.4	83.1	76.8	76.9	68.5	76.3
Ceresan	0.25	75.9	76.6	73.5	80.2	75.7	76.2	69.2	75.3
Agrosan GN	0.30	77.6	76.5	71.7	79.5	77.0	75.5	69.2	75.3
Tillex	0.25	75.6	74.0	73.5	78.6	77.0	73.0	68.9	74.4
Copper Carbonate	0.30	79.4	70.6	71.1	80.2	72.7	72.8	69.0	73.7
Fusariol	0.25	72.6	74.9	70.0	78.3	76.2	74.8	60.7	72.5
Phygon	0.20	74.3	73.9	68.6	79.3	74.3	73.2	62.4	72.3
Flit 406	0.25	64.4	67.9	66.7	79.5	71.7	60.7	53.4	66.3
Control	—	76.8	72.9	68.3	77.7	75.2	73.3	64.3	72.6
Mean		75.4	74.4	70.8	79.7	75.5	73.1	65.8	

C. D. at 5 per cent level for :—

Treatments	=	2.9
Containers	=	1.6
Years	=	1.9
Treatment × Containers	=	5.1
Treatment × Years	=	5.9

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Treatment × Years: There was no significant reduction in the viability of seeds treated with Fernasan, Ceresan, AgrosanGN, Fusariol and in that of the untreated control even after storage for a period of two years.

The results of present investigations clearly indicate that a higher level of germination is recorded when wheat is treated with Ceresan, and barley with Fernasan than that in the untreated controls. Koehler and Bever[2] (1956) obtained similar results by treating wheat with organo-mercurials.

Germination was significantly higher when wheat seed treated with Fusariol, Phygon and Flit 406 were stored in alkathene lined bags as compared with gunny bags or air-tight bottles. Richards[3] (1953) has reported that alkathene has very low water vapour and high organic vapour permeability. Lower germination in air-tight bottles may be due to toxic affect of vapours on seeds which can easily pass out from alkathene-lined bags and ordinary gunny bags.

It is evident that wheat and barley seed treated with Ceresan (0.2 per cent) and Fernasan (0.3 per cent) respectively, can be stored in air-tight containers or alkathene- lined bags for a period of one year without any significant loss of viability and furthermore, treated seed will give higher germination than the untreated seed.

SUMMARY

Wheat and barley seeds treated with nine fungicides were stored in three types of containers, e.g. air-tight bottles, alkathene-lined gunny bags and ordinary gunny bags and their germination was tested one day before storage and at the end of one, two and three years' storage at room temperature (15° C to 40° C).

The viability of wheat seeds treated with Ceresan, Copper carbonate, Fernasan, Agrosan GN, Tillex and Agrosan 5W was significantly higher, while that of seeds treated with Fusariol, Phygon and Flit 406 treated was significantly lower than that in the untreated control when stored in air-tight bottles. Seeds treated with Fusariol, Phygon and Flit 406 however, gave significantly higher germination when stored in alkathene-lined gunny bags than those stored in the other two types of containers.

Viability of barley seeds was significantly higher in Fernasan and Agrosan 5W treatments and significantly lower in Flit 406 treatment as well as untreated seeds. Highest viability of wheat seeds was, however, recorded in the case of Ceresan treatment and that of barley in Fernasan treatment at the end of three years' storage.

---

[2]Koehler, B. and W. M. Bever (1956) *Pl. Dis. Reptr.* 40 : 490.

[3]Richards, R. B. (1953) Kirk othewer-encyclopedia chemical Technology, 10 : 1938.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ACKNOWLEDGEMENTS : Thanks are due to Dr. B. L. Chona the former head and Dr. S. P. Raychaudhuri present Head of the Division of Mycology and Plant Pathology, for keen interest and helpful suggestions during the course of this investigation. We are grateful to Mr. S. S. Rohewal, Assistant Geneticist, Indian Agricultural Research, Institute, for the immense help in the statistical analysis of the data.

Division of Mycology and Plant Pathology

Indian Agricultural Research Institute

New Delhi-12

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STATUS OF LINSEED-RUST RACES IN INDIA AND SOURCES OF RESISTANCE

D. P. MISRA* AND R. PRASADA

(Accepted for publication May 5, 1966)

Information on the race-flora of linseed rust (*Melampsora lini* (Ehrenb.) Lév.) prevalent in India during the 16 years ending 1962 has been supplied by Prasada (1948), Sethi, Misra, Lele and Joshi (1962), and Misra and Lele (1963). Since the reactions incited by the six physiologic races found to occur in India are different from those described in the International key (Flor, 1954), these have been designated as I-1 (Indian-1) to I-6. In the present paper, the race-flora observed during the subsequent years, *i.e.* 1963 and 1964, has been given. This includes the finding of a new race designated I-7.

Prasada (1958) and Misra (1964 a) have reported the reactions of a large number of varieties of *Linum usitatissimum* and species of *Linum* against the prevalent races of the rust. In the studies reported herein, this material has also been screened against the new race I-7. Besides this, a number of additional linseed varieties and other species of *Linum* were tested with all the known races of *M. lini*. An up-to-date list of rust-resistant material is presented.

By reviewing the data of the physiologic-race analysis obtained during the last 18 years and the available sources of rust resistance, it is possible to plan future work to increase yields of linseed through systematic breeding.

RESULTS : *Race-flora during 1963 and 1964* : During 1962-63 and 1963-64, 111 rust samples from different parts of the country were analysed. In addition to the six races previously known to occur in India, a new race (I-7) was met with in a 1963-64 collection from U.P. The distribution of races in different States has been summarised in Table 1.

On the basis of the virulence of races on differential hosts, races I-1 and I-3 are the weakest and differ from each other in their reactions on Wilden and Williston Brown. These races were found to be most common and constituted 67 per cent of the isolations studied. The next race, in order of prevalence, was I-4, which was present in 17 per cent of the isolations. Race I-4 infects the differential variety Burk, in addition to those susceptible to the races I-1 and I-3. Race I-5, to which Burk is resistant and Bison is susceptible, was represented in 10 per cent isolations. The remaining three races, I-2, I-6 and I-7 (new) together, were present in less than 6 per cent isolations.

*Mycologist, Botanical Sub-station, Indian Agricultural Research Institure, Pusa Bihar.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

TABLE 1 : Occurrence and distribution of races of *M. lini* during 1963 and 1964

State	Number of times each race picked up from the crop-year												
	1962-63						1963-64						
	I-1	I-2	I-3	I-4	I-5	I-6	I-1	I-2	I-3	I-4	I-5	I-6	I-7*
Himachal Pradesh	2	—	1	—	—	—	20	1	8	6	2	1	—
Punjab	2	1	2	—	—	1	6	—	5	—	2	1	—
Delhi	—	—	—	—	—	—	4	—	2	5	1	—	—
Uttar Pradesh	2	—	5	2	1	—	13	—	8	3	3	—	1
Bihar	—	—	—	—	—	—	1	—	—	1	1	—	—
Orissa	1	—	2	2	2	—	—	—	—	—	—	—	—
Assam	—	—	—	1	—	—	—	—	—	—	—	—	—
Madhya Pradesh	—	—	—	—	1	—	—	—	—	2	—	—	—
Total isolations	7	1	10	5	4	1	44	1	23	17	9	2	1
	Twenty-eight (28)						Ninety-seven (97)						

Testing of varieties of linseed and species of Linum : Out of 331 varieties of *L. usitatissimum* and 20 species of *Linum* (including exotics from Afghanistan, Australia, Argentina, Ireland, Latvia, Sweden and U.S.A.), material resistant to all the seven Indian races of linseed rust is listed below.

Exotic varieties of linseed : L.I. 5628, L.I. 5691, Russian type 36, Afghanistan 2, Charrna M.A., Versaille 32 (yellow seed), Versaille 32 (grey seed), K. 5835, W. 5039 CC, W. 6060 DD, W. 6063 DD, W. 6064 DD, W. 4108 EE, W. 5214 EG, W. 2392 JU (Vide Hd.B., I.A.R.I.'s No. RT/61-78), Avondale 2, Beladi Y 6420 Sel. SB 43-D-I, Punjab × Walsh × Punjab × NSW/948, P 3118 (Moose × N. Dakota), P. 3655, P. 3709, P. 4194 (D. 83), Jogeva 108, Very Pale Blue Crimped 97, Bewing 202, Entre Rios, D. 83, 806/3, Ghabreh, Marmanko, Pshoff, Punjab Selec. 39-21-1, Big Moose × Redwing Y 6202, Morocco G/38/1671, North Dakota, Rio D, Rio × concument, Ottawa 770B, Dakota, Cass, Clay, Polk, Birio, Kenya, Abyssinian, Leona, Victory-A, Bowman, Norman, Pale Blue Crimped, Bolly Golden Selection, Grant, Towner, Morye, New Land, Italia Roma, Tames Blue Crimped, Punjab 90 (Vide Hd. B., I.A.R.I.'s No. RT/61-101) and Marshall.

*New race.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Indian varieties oflinseed: NP (RR) 9 (Vide Hd. B., I.A.R.I.'s No. RT/61-28), NP (RR) 10, NP (RR) 56, NP (RR) 95, NP (RR) 218, NP (RR) 279B, NP (RR) 279K3, NP (RR) 368, NP (RR) 381, NP (RR) 389, NP (RR) 415, NP (RR) 501, I.A.R.I. Selec. 192-5 and I.A.R.I. Selec. 208 B-2.

Species of Linum: *L. africanum*, *L. alpinum*, *L. angustifolium*, *L. corymbiferum*. *L. creptans*, *L. flocossum*, *L. gallicum*, *L. grandiflorum*, *L. hispidum*, *L. hologynum*, *L. marginale*, *L. odoratum*, *L. perenne*, *L. tenke*, *L. tennue* and *L. trigyna*.

Varieties of linseed which were not resistant to most of the races are of little practical utility in the breeding programme and have not been mentioned here. They have, however, been listed by Misra (1964 a) and could be utilised for incorporating resistance to specific races.

Report of a new race of M. lini : As has been mentioned above, a new race of linseed rust was picked up from the crop year 1963-64. A rust sample from Agra on variety S. 36 produced the susceptible type of reaction on differential host Barnes, which was resistant to the races picked up in India till then. A single-spore culture was, therefore, established from a pustule on Barnes. The reactions produced by this culture on the set of standard differentials were similar to those produced by race I-4, except that Barnes was susceptible to the new race which has been designated as race I-7. To the new race, in the differential hosts, Ottawa 770B, Bakota, Cass, Clay, Polk, Birio, Kenya, Bison, Ward, Koto, Abyssinian, Grant, Minnesota Selection, Leona, Victory-A, Towner, Marshall, Bowman, Pale Blue Crimped, Wells, Norman, Bolly golden Selection and Cortland are immune ; Bombay, Stewart, Akmolinsk, Williston Brown, Burk and Barnes are susceptible and Wilden Semi-resistant.

DISCUSSION : The prevalence of races of *Melampsora lini* during 1963 and 1964, as seen from the present studies, appeared to follow the same trend as reported in the previous 16 years in the country (Sethi, *et al*, 1962 ; Misra and Lele, 1963). However, regarding the identification of races, there are important considerations, which are reviewed.

During the course of race determinations since 1947, changes were made in the differential varieties. Therefore, the identity of races for the entire period is not precisely comparable. To quote an instance, race I-7 which was picked up in a rust collection from 1964 crop, could not have been distinguished from race I-4, since Barnes was not used till 1958. Further, most of the collections were studied on differentials which are reported to be apparently monogenic for rust in U.S.A., and each differential is reported to carry one of the multiple alleles in either of the five loci named as K,L,M,N and P (Flor, 1955). While selecting the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

differentials, Bison—a universal suscept—was back-crossed. Later, Bison was found to be resistant to some of the races in Australia and it was determined to carry L_9L_9gene. In India, the presence of additional L_9L_9 gene was detected in some of the differentials, known to be monogenic for alleles in K,M, L and P (Misra, 1964 b). It has also been found that such varieties are resistant to races avirulent on Bison, irrespective of the reactions, as a result of the presence of other genes in them. It implies, therefore, that the differentiation of such races could have been obliterated in the past, in relation to differentials carrying additional Bison-gene (L_9L_9). While it may be reasonable to evolve monogenic differentials for the country to know the precise race-picture in future, it would be worth while to group the races observed in the past, on the basis of reactions on Bison, which has been in use from the very beginning. Thus, races I-1, I-3, I-4 and I-7 constitute group A, (Bison resistant) and races I-2, I-5 and I-6 constitute group B, (Bison susceptible). The scrutiny of 18 years' (1947-1964) data of race analysis of 1066 isolations shows that the race group A was more prevalent, with 79 per cent isolations, than race group B, comprising only 21 per cent isolations. Independently, in any region of the country and in any crop-year, group A was more common. It is interesting that races of group A infect a smaller number of monogenic differentials than those of group B. In this connection the following statement by Flor (1956) has an important bearing. This reads, 'In a region where the sexual stage initiates each year's infection, there has been no tendency towards the development of races of wider virulence than required to attack cultivated varieties The tendency has been towards the loss of unnecessary genes for virulence.' Such a phenomenon may have been in operation in India, where the annual initiation of the rust through sexual stages has been found to occur and cultivation of highly susceptible linseed varieties has continued since the unknown past (Misra, 1961 ; Misra and Prasada, 1965). The prevalence of races in India substantiates the aforesaid view expressed by Flor.

In the light of these considerations, it cannot be assumed that the race-picture will continue to be as before, when in future the resistant host material is widely dispersed for cultivation. At present there does not seem to be any paucity of donors for rust-resistance, but these should be judiciously used. It has been found by Misra (1964b, 1966) that *Linium usitatissimum* varieties Grant, Clay, Birio, Norman, Bolly Golden Selection, Pale Blue Crimped, Minnesota Selection, Towner, Abyssinian, Wells ; *L. gallicum*, *L. africanum*, *L. angustifolium*, *L. strictum*, *L. marginale* and *L. flocossum* carry single genes, which condition resistance to all the races in India. In case these genes are incorporated in the commercial varieties, the breeding procedure will be simplified. Besides, it would be possible to reorientate the programme quickly in case new pathogenic races for particular genes are found in future.

SUMMARY

Analysis of 111 rust samples from 1962-63 and 1963-64 crops yielded 6 known races and one new race. The reactions of the new race are given. Prevalence of races during these yeare showed similar trend,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

as had been found in the previous 16 years. 73 varieties of linseed and 17 species of *Linum* found to be resistant to all the 7 races have been listed. These were out of 331 varieties and 20 species of *Linum* tested. The present position of race-flora in India and the utilisation of the sources of resistance have been discussed.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B.L. Chona, the former head and Dr. S. P. Raychaudhry, the present Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for their keen interest in the work.

Indian Agricultural Research Institute
Plant Pathological Sub-Station
Flowerdale, Simla-East

REFERENCES

FLOR, H.H. (1954) Identification of races of flax rust by lines with single rust-conditioning genes. U.S.D.A., *Tech. Bull.*, **1087** : 1-25.

FLOR, H.H. (1955) Host—parasite interaction in flax rust, its genetics and other implications. *Phytopathology* **45** : 680-685.

FLOR, H.H. (1956) The complementary genic system in flax and flax rust. *Advances Genet.*, **8** : 29-54.

MISRA, D.P. (1961) Natural occurrence of pycnial and aecial stages of linseed rust, *Melampsora lini* (Pers.) Lev. in India. *Abs. 2nd. Oilseeds Workers Conf. I.C.O.C.*, Madras.

MISRA, D.P. (1964a) Rust resistance in the species and varieties of *Linum* against Indian physiologic races of linseed rust. *Indian Oilseeds J.*, **8** : 277-279.

MISRA, D.P. (1964b) Genes conditioning resistance of Flor's differentials to Indian races of linseed rust. *Indian J. Genet.*, **24** : 227-238.

MISRA, D.P. (1966) Genes conditioning resistance of *Linum* species to Indian races of linseed rust. *Indian J. Genet.* (In press).

MISRA, D.P. AND V.C. LELE (1963) Physiologic races of linseed rust in India during 1960, 1961 and 1962—prevalence and distribution. *Indian Oilseeds J.*, **7** : 336-37.

MISRA, D.P. AND R. PRASADA (1965) Linseed Rust can be controlled. *Indian Farm.*, **15** : 9-10.

PRASADA, R. (1948) Studies on Linseed Rust, *Malampsora lini* (Per.) Lev. in India. *Indian Phytopath.* **1** : 1-8.

PRASADA, R. (1958) Rust resistance in Linseed varieties against Indian Physiologic Races of *Melampsora lini* (Pers.) Lev. Proc. First Confr. Oil-seed Wkrs. in India, Chandigarh, 1958.

SETHI, C.L., D.P. MISRA. V.C. LELE AND L.M. JOSHI (1962) Occurrence of physiologic races of linseed rust in India (1946-1959). *Indian Phytopath.* **15** : 207-210.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

STUDIES ON A MOSAIC DISEASE OF PEA (PISUM SATIVUM L.)

T. N. SREENIVASAN AND T. K. NARIANI

(Accepted for publication March 14, 1966)

INTRODUCTION : During the past few years peas (*Pisum sativum* L.) grown at the Indian Agricultural Research Institute, New Delhi, and in the home gardens around the Institute have been found to be affected with a mosaic disease. Up to 10 per cent incidence of the disease was recorded in some of the varieties. As the symptoms of the disease were suggestive of virus origin, investigations were conducted on the mode of transmission, host range and physical properties of the causal virus. The results of the investigations have been presented in this paper.

MATERIAL AND METHODS : The culture of the virus was obtained from the pea variety Bonneville, naturally infected with mosaic disease and was maintained by sap inoculations to healthy pea and sannhemp (*Crotalaria juncea* L.) plants. For all experiments, pea variety Bonneville was used as a test plant. For insect transmission tests, virus-free cultures of the various aphid species and white flies (*Bemisia tabaci* Gen.) were used.

Field symptoms : The diseased plants are pale, weak and dwarfed. The young leaves and stipules show a general mosaic mottling whereas the older ones display distinct irregular whitish areas (Fig. 1). The tendrils are curled abnormally and the apical leaves remain folded. Invariably small specks of light brown discoloured areas are seen on petioles, stem and tendrils. The diseased plants flower later than the healthy ones and the flowers are smaller in size and fewer in number. Comparatively fewer pods are set on diseased plants and they are often smaller in size and contain a few seeds.

Transmission of the disease : *Mechanical sap inoculation* : The disease was transmitted to healthy pea plants by mechanical inoculation with the infective sap prepared from diseased pea plants. The symptoms of the disease appeared usually after five days during March and April and after 9-10 days during the colder months, *i.e.* December and January.

Insect transmission : Six species of aphids viz. *Aphis craccivora* Koch., *A. gossypii* Glov., *A. evonymi* Fabr., *Myzus persicae* Sulz., *Rhopalosiphum pseudobrassicae* (Davis) and *R. maidis* Fitch. were tested for their ability to transmit the virus. The aphids were starved for one hour and fed on diseased pea leaves for ten minutes. Batches of ten aphids from these were then transferred to each test plant. The aphids were left on the test plants for 24 hours after which they were removed and the plants sprayed with 0.1 per cent Ekatox solution. All the six species of aphids tested transmitted the disease. The virus, however, could not be transmitted by white flies (*Bemisia tabaci* Gen.).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Seed transmission tests : Pea seeds were collected from mosaic-infected plants from the field as well as from artificially infected plants in theglass house and sown in pots in the insect-proof glasshouse. None of the 1188 pea plants raised in this manner developed disease symptoms, indicating thereby that the virus is not transmitted through seed.

Seed transmission tests were also conducted with seeds collected from artificially infected plants of *Vicia faba* L. and *Trigonella foenum graecum*. Out of 144 and 501 plants of *Vicia faba* and *Trigonella foenum graecum* none was found to be diseased.

Host range : The host range of the virus was determined by attempted transmission to 97 plant species belonging to 15 different families. Inoculations were made on the young leaves of vigorously growing plants. The plants which did not exihibit visible symptoms were indexed on healthy pea plants to determine if they were symptomless carriers of the virus.

The virus was transmitted by sap inoculation to *Pisum sativum* L. var. *arvense*, *Vicia*, *faba* L. (Fig. 2), *V. narbonensis* L., *V. sativa* L., *V. biennis*, *Lathyrus odoratus* L., *L. ochrus*, *L. sativus*, *Trigonella foenum-graecum* L., *T. corniculata*, *Crotalaria juncea* L., *Lupinus augustifolius* L., *L. albus* L., *Cicer arietinum* L. and *Trifolium subterraneum*. *Chenopodium amaranticolor* Coste and Reyn reacted with local chlorotic spots on the inoculated leaves (Fig. 3). The symptoms produced on *Lupinus augustifolius* L. were characteristic of the 'sore-shin' disease (Fig. 4). A dark brown discolouration appeared on the stem just below the apex and the stem bent into a curl. The leaves started wilting from above till the whole plant wilted. The following plant species were found to be non-susceptible.

Vicia villosa Roth., *Phaseolus vulgaris* L., *P. lunatus* L., *P. aconitifolius* Jacq., *P. calcaratus* Roxb., *P. trilobus* L., *P. mungo* L., *P. aureus* Roxb., *Dolichos lablab* L., *D. biflorus* L., *Soja max* Piper, *Canavalia ensiformis* D C., *Lens esculenta* Moench., *Cajanus cajan* (L.) Millsp. *Crotalaria sericea* Retz., *C. verrucosa* L., *Cyamopsis tetragonoloba* (L.) Taub., *Clitoria terneata* L., *Vigna sinensis* Savi., *Melilotus alba* Desr., *M. indica* All., *Arachis hypogaea* L., *Trifolium pratense* L., *T. glomeratum* L., *T. hybridum* L., *T. repens* L., *Desmodium limense*, *D. virgatus*, *Medicago sativa* L., *Cassia tora* L., *Nicotiana tabacum* L. var. Harrison Special, *N. tabacum* var. White Burley, *N. rustica* L., *N. glutinosa* L., *Capsicum annuum* L., *Solanum melongena* L., *S. nodiflorum* L., *S. nigrum* L., *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Petunia hybrida* Vilm., *Helianthus annuus* L., *Lactuca sativa* L., *Tagetes* sp., *Chrysanthemum indicum* L., *Zinnia elegans* Jacq., *Dahlia* sp., *Amaranthus gangeticus* L., *Gomphrena globosa* L., *Brassica oleracea* L., var. *capitata* L., *B. oleracea* L. var. *botrytis* L., *B. caulorapa* Pasaq., *B. campestris* L. var. *sarson* Prain., *B. juncea* L., *B. rapa* L., *Daucus carota* L., *Abelmoschus esculentus* (L.) Moench., *Althaea rosea* L., *Impatiens balsamina* L., *Ipomea* sp., *Sesamum indicum* L., *Euphorbia hirta* L., *Racinis communis* L., *Chenopodium album* L., *C. murale* L., *Spinacea oleracea* L., *Fragaria vesca* L., *Cucurbita maxima* Doch., *Cucumis sativus* L., *C. melo* L., *Lagenaria siceraria* Standl. var. long, *Citrullus vulgaris*, Schrad,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Momordica charantia L., *Trichosanthes anguina* L., *Ocimum sanctum* L., *Vinca rosea* L., *Oryza sativa* L., *O. glaberrima* Stand., *Triticum aesativum* L., *Hordeum vulgare* L.,*Zea mays* L.

Physical properties :

The virus in the standard extract was infective after heating to 58°C for ten minutes but was rendered innocuous at 60°C and had a dilution end point between 1 : 1000 and 1 : 2000. The longevity *in vitro* of the virus was found to be 24—42 hours at room temperature (19°—27°C), 66—72 hours at 14° —16°C and 72—90 hours at 8°—10°C. The virus was found to be infective in diseased leaves desiccated at room temperature (19°—30°C) in a desiccator over calcium chloride for 144 hours but not after 168 hours. It had an optimum pH infection range from 6—8.

The virus was found to be present and could be recovered from all the plant parts of infected plants except the root nodules and the seeds. Pea plants of different ages at all the stages of growth were found to be susceptible to infection.

Varietal reaction : Thirty two varieties of pea, viz. Ace. Advancer, Alderman, Alfer, Alaska, Asauji, Bonneville, Bountiful, Canners Perfection, Dwarf Sugar, Dwarf Telephone, Early Giant, Early Perfection, First and Best, Giant Stride, Giant Sugar poded, Greenfeast, Horal, Hundredfold, Kalanagini, Kelvedon Wonder, Lincoln, Laxton's Superb, Little Marvel, Morses Market, Nott's Excelsior, N.P. 29, Premium Gem, Rimpus, Senator, Sylvia and Thomas Laxton were tested for their reaction to the pea mosaic virus. Inoculations were made on the leaves of 2 to 3 week old seedlings by rubbing the infective juice with carborundum powder.

Pea varieties Canners Perfection, Horal, Hundredfold and Little Marvel were found to be immune to infection. The varieties Dwarf Sugar and Kelvedon Wonder were found to be highly resistant. The rest were susceptible. The immunity to infection of the varieties Canners Perfection, Horal, Hundredfold and Little Marvel was further confirmed by feeding viruliferous aphids (*Aphis craccivora* Koch.) on them. None of the plants tested in this manner were infected.

DISCUSSION : Mosaic diseases of pea have been reported by several workers since the first report by Dickson (1922) from Canada. Detailed studies have been made by Osborn (1938), Pierce (1935, 1937), Murphy and Pierce (1937), Stubbs (1937), Ainsworth (1940), Zaumeyer (1940), Chaudhuri (1950), Hagedorn and Walker (1954), Goodchild (1956) and Klesser (1960). As a result of these studies five different viruses, namely, Pea mosaic virus 1 (Pea enation mosaic virus), Osborn's Pea mosaic virus 2, Pea mosaic virus 3 (common pea mosaic virus), Pea mosaic virus 4 and Pea mosaic virus 5 (pea stunt mosaic virus) have been found to be associated with mosaic symptoms on pea.

The virus under study differs from Pea enation mosaic virus in some physical properties and the absence of characteristic leaf enations. Pea enation mosaic virus is also reported to be infectious to all the pea varieties including Perfection and Horal. Osborn's Pea Virus 2 differs from the virus reported herein in physical properties as well as in its

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ability to infect *Phaseolus vulgaris*. The pea mosaic virus studied here also differs from pea mosaic virus 4 and 5 in physical properties and host range. The Pea mosaic virus 3 or common pea mosaic virus designated as *Pisum virus* 2 or *Marmor leguminosarum* has been differentiated by Stubbs (1937) into three strains, namely, marble pea mosaic (Pea virus 2A), speckle pea mosaic (Pea virus 2B) and mild pea mosaic (Pea virus 2C) based on symptomatology and their non-transmissibility to red clover (*Trifolium pratense*). Azad *et al* (1961) reported a new strain of the virus from *Vicia faba* and designated it as *Pisum virus* 2D on account of its transmissibility to soybean (*Soja max*). The virus under study differs from *Pisum virus* 2D as it does not infect *Soja max*, *Vicia villosa* and *Trifolium pratense* but infects *Lupinus augustifolius*. It resembles in physical properties, host range, varietal reaction and aphid transmission the common pea mosaic virus which is also known to cause the sore-shin disease in lupin (*Lupinus augustifolius*) (Chamberlain 1935) and more closely the marble pea mosaic strain Pea virus 2A of Stubbs as it is not transmissible to red clover (*Trifolium pratense*).

Summary

A sap transmissible mosaic disease of pea (*Pisum sativum* L.) has been found to be transmissible by the aphids, *Aphis craccivora* Koch., *A. gossypii* Glov., *A. evonymi* Fabr., *Myzus persicae* Sulz., *Rhopalosiphum pseudobrassicae* (Davis) and *R. maidis* Fitch, but not through the seed of infected pea, *Vicia faba* or *Trigonella foenum-graecum*.

The virus was transmitted to *Pisum sativum* var. *arvense*, *Vicia faba* L., *V. narbonensis*, *V. sativa*, *V. biennis*, *Lathyrus odoratus* L., *L. ochrus* L., *L. sativus*, *Trigonella foenum-graecum*, *T. corniculata*, *Crotalaria juncea*, *Lupinus albus*, *L. augustifolius* and *Cicer arietinum*. *Chenopodium amaranticolor* produced countable local chlorotic lesions on the inoculated leaves. The virus had a thermal inactivation point of 58°—60°C and a dilution end point beween 1 : 1000—1 : 2000. The longevity *in vitro* of the virus was found to be 24--42 hours at room temperature (19°—27°C), 66--72 hours at 14°—16°C and 72—90 hours at 8°—10°C. The virus was found to be infective in diseased leaves desiccated at room temperature for 144 hours but not after 168 hours. It had an optimum pH infection range from 6—8. Pea varieties Canners Perfection, Horal, Hundredfold and Little Marvel were immune to infection with the virus. The causal virus has been identified as the strain 2A of the common pea mosaic virus (*Pisum virus* 2 Smith or *Marmor leguminosarum* Holmes).

Acknowledgement : The authors are grateful to Dr. S. P. Raychaudhuri and Dr. R. S. Vasudeva the present and the former Head of the Division of Mycology and Plant Pathology respectively, for their suggestions and interest during the course of this investigation.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute
New Delhi

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

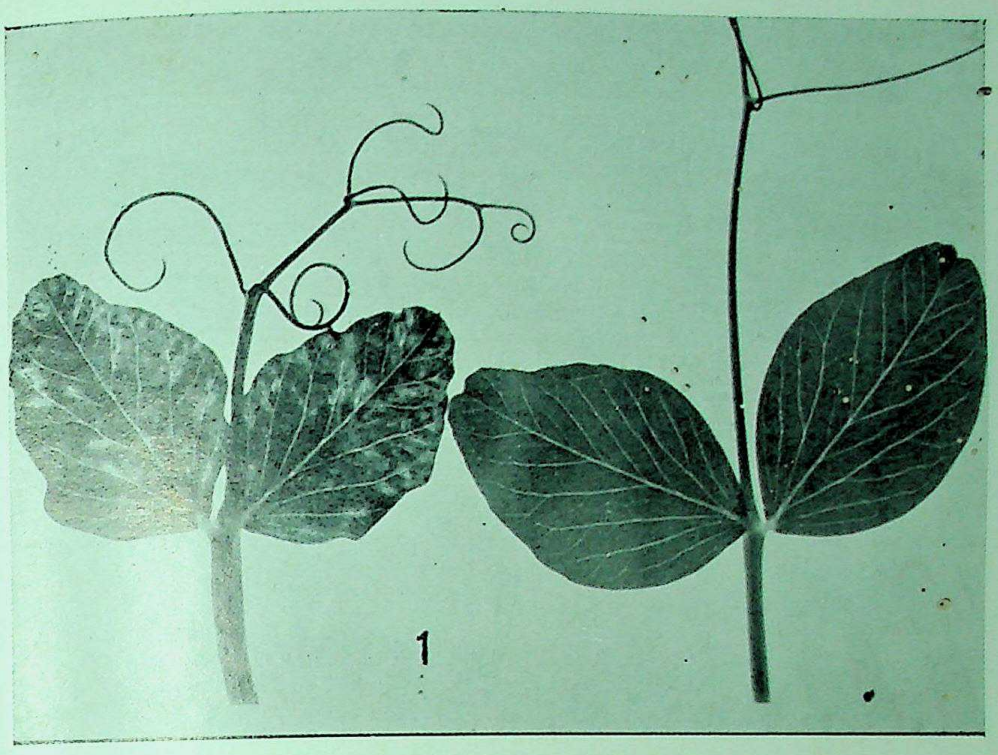


Fig. 1. Healthy and mosaic affected leaves of pea (*Pisum sativum* L.)

Fig. 2. Mosaic symptoms on *Vicia faba* L.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

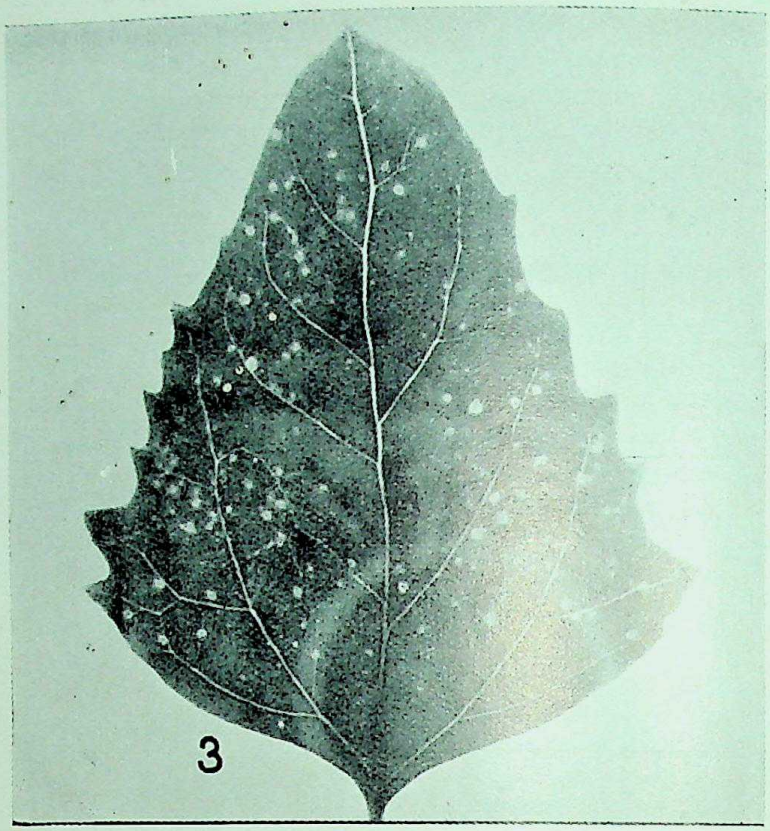


Fig. 3. *Chenopodium amaranticolor* showing chlorotic local lesions.

Fig. 4. *Lupinus augustifolius* showing symptoms of "sore-shin" disease

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

REFERENCES

AINSWORTH, G. C. (1940) The identification of certain viruses found infecting leguminous plants in Great Britain. *Ann. appl. Biol.*, **27** : 218-226.

AZAD, R. N., B. B. NAGAICH, AND O. P. SEHGAL (1961) Broad bean mosaic virus in India. *Indian Phytopath.* **14** : 169-173.

CHAMBERLAIN, E. E. (1935) Sore-shin of blue Lupins. Its identity with pea mosaic. *N. Z. J. Agric.*, **51** : 86-92.

CHOWDHURI, R. P. (1950) Studies on two aphid transmitted viruses of leguminous crops. *Ann. appl. Biol.*, **37** : 342-354.

DICKSON, B. T. (1922) Studies concerning mosaic diseases. *Mac Donald College Tech. Bull.*, **2** : 125 pp.

GOODCHILD, D. J. (1956) Relationships of legume viruses in Australia I. Strains of bean yellow mosaic virus and pea mosaic virus. *Aust. J. Biol. Sci.*, **9** : 213-230.

HAGEDORN, D. J. AND J. C. WALKER, (1954) Virus diseases of canning peas in Wisconsin. *Res. Bull. Wis. agric. Exp. Sta.*, **185** : 32 pp.

KLESSER, P. J. (1960) Virus diseases of peas and sweet pea. *Bothalia*, **7** : 253-282.

MURPHY D. M. AND W. H. PIERCE (1937) Common mosaic of the garden pea, *Pisum sativum. Phytopathology* **27** : 710-721.

OSBORN, H. T. (1938) Studies on Pea virus 1. *Phytopathology* **28** : 923-934.

PIERCE, H. W. (1935) The identification of certain viruses affecting leguminous plants. *J. agric. Res.*, **51** : 1017-39.

PIERCE, W. H. (1937) Legume viruses in Idaho. *Phytopathology* **27** : 836-843.

STUBBS, M. W. (1937) Viroses of garden pea. *Phytopathology* **27** : 242-266.

ZAUMEYER, W. J. (1940) Three previously undescribed mosaic diseases of pea. *J. Agric. Res.*, **60** : 433-452.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

ESTIMATION OF LOSSES DUE TO GROUND-NUT MOSAIC

V.V. CHENULU, R.L. MUNJAL, T.S. HORA AND AMAR SINGH

(Accepted for publication February 28, 1966)

A mosaic disease of groundnut (*Arachis hypogaea L.*) was first observed in 1949 on the farm area of the Indian Agricultural Research Institute, New Delhi, wherein nearly 40-50 per cent of the plants were affected and resulted in an appreciable loss to the crop. *Nariani and Dhingra (1963) described the symptoms of the disease and established its viral nature by graft transmission. The mosaic disease of ground-nut has been found to differ from 'Rosette' in its failure to be transmitted by mechanical means or by the aphid vector *Aphis craccivora* Koch. Since the reported occurrence of the disease in 1949 it is found every year in the ground-nut crop raised in the Division of Agronomy, Indian Agricultural Research Institute. In view of the regular recurrence of the disease affecting 40-50 per cent of the plants and reducing yields considerably, experiments had been undertaken since 1958 to find out the disease-intensity-yield-loss relationships and the results obtained are reported in this paper.

MATERIAL AND METHODS : A highly susceptible, commonly grown variety of groundnut, Punjab 1, was sown in the Agronomy Division of this Institute in the last week of May or early June. The incidence of the disease was recorded at fortnightly intervals. Observations revealed that the disease affected some plants more severely and others less, and that the severity of disease depended on the early or on late appearance of the disease and on the age of the crop. In order to distinguish plants affected with different degrees of disease intensity, five grades viz A (healthy); B (25 per cent infection), C (50 per cent infection), D (75 per cent infection) and E (100 per cent infection) were set up. The description of different disease intensity grades is detailed below.

Grade A

(*Healthy*) : Plants showing normal growth with an average height of 24 cm., with a radial diameter of 88-92 cm. and having an average of 9 main branches and 4-5 sub-branches for each main branch.

Grade B

(*25 per cent*) : Plants appearing normal but with one or two top-most leaves of a few branches showing characteristic mosaic mottling and smalling, with an average height 22 cm., and ardial diameter 74-82 cm., main branches 9 with 4 subbranches for each.

*Nariani, T.K. and K.L. Dhingra (1963). Indian Jour. Agr. Sci., 33, 25-27.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Grade C

(50 per cent) : Almost all the branches of an affected plant are diseased. Each infected branch bears a few normal leaves accompanied by small, crinkled leaves. There is a visible reduction in the size of the plant as also in the length of its branches. The average height is 19 cm., radial diameter 51-57 cm.,main branches 8 with 8-9 sub-branches for each.

Grade D

(75 per cent) : The plants are conspicuous by their small size and bushy appearance. All the branches of the plant are affected. The plants rarely have fully formed normal leaves. The twig length is very much reduced. The branches and sub-branches are all pressed on a short axis giving it a typical bunchy appearance. The average height is 13 cm., the radial diameter 22-28 cm., main branches 6 with 5-6 sub-branches for each. Affected plants do not normally bear pods, and those pods that are formed do not have fully developed kernels.

Grade E

(100 per cent) : These plants almost resemble grade 'D' plants, except that the reduction in plant size is much more prominent. All the leaves of all the branches are reduced in size, the branches are also very much shortened. The average height is 11 cm., the radial diameter 17-20 cm., main branches 5 with 4-5 sub-branches for each. Plants of this category generally do not bear any pods and this results in almost 100 per cent loss in yield.

Plants of different grades can be seen in Plate 1, Figs. A, B, C, D and E respectively. One hundred plants in each of the disease -intensity categories were selected at random in the field when the crop was in full bloom and pod-setting had just commenced. This stage was chosen for tagging the plants because losses are expected to be maximal and directly proportional to the disease intensity at this stage. At the time of the harvest individual plant yields in terms of pod weight as well as kernel weight of all the tagged plants were recorded. In the earlier years viz., 1958, 1959, the green weight of the plants cut at the ground level was also taken to find out if any correlation existed between the green weight and the yield. It was, however, observed that there was no such correlation. Accordingly, the recording of data on the green weight of plants was dropped out in subsequent years.

EXPERIMENTAL RESULTS : The experiment to determine the relationship between disease intensity and yield loss due to groundnut mosaic was repeatedfor six years from 1958 to 1963. During all these years, the crop was sown in the same plot (Plot 2B-South, Agronomy Division of Indian Agricultural Research Institute) and the same susceptible variety Punjab-1 was used. Data in respect of loss in yield due to different disease

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

intensities in different years are set out in Table 1. Data for the year 1961 were deleted as the experiment was vitiated by unusually heavy rains in July-August affecting normal flowering and pod setting. The per cent loss in yield due to different disease intensity categories was calculated according to the following formula :

$$\text{Per cent loss in yield} = \frac{100 \times (\text{Average yield of healthy plant} - \text{Average yield of diseased plant})}{\text{Average yield of healthy plant}}$$

TABLE 1 : Loss in yield of groundnut due to different disease intensity levels of mosaic in the variety Punjab-1 in different years.

Disease intensity (Grade)	1958		1959		1960		1962		1963	
	Per cent reduction		Per cent reduction		Per cent reduction		Per cent reduction		Per cent reduction	
	Pod wt.	Kernel wt.	Pod wt.	Kernel wt.	Pod wt.	Kernel wt.	Pod wt.	Kernel wt.	Pod wt.	Kernel wt.
B (25 per cent)	40	37	31	35	28	30	22	29	48	47
C (50 per cent)	72	73	57	58	61	64	73	74	85	86
D (75 per cent)	90	89	75	75	81	82	92	93	97	97
E (100 per cent)	97	97	96	93	92	92	99	97	100	100
Regression coefficient	28.0	27.9	25.1	24.9	25.4	25.7	28.0	28.1	30.3	30.3

From the results set out in Table 1, it is seen that the loss in yield in terms of pod weight varied from 22to48 per cent in grade B, 61to85 per cent in grade 'C' 75to97 per cent in grade D and 92to100 per cent in grade E in different years. In terms of kernel weight the loss in yield from year to year varied from 29to47 per cent in grade B, 57to86 per cent grade C, 75to97 per cent in grade D and 92to100 per cent in grade E respectively. Thus the results are more or less similar whether expressed in terms of pod weight or kernel weight. The loss in yield of grade D and grade E plants was always more than 75 and 92 per cent in different years.

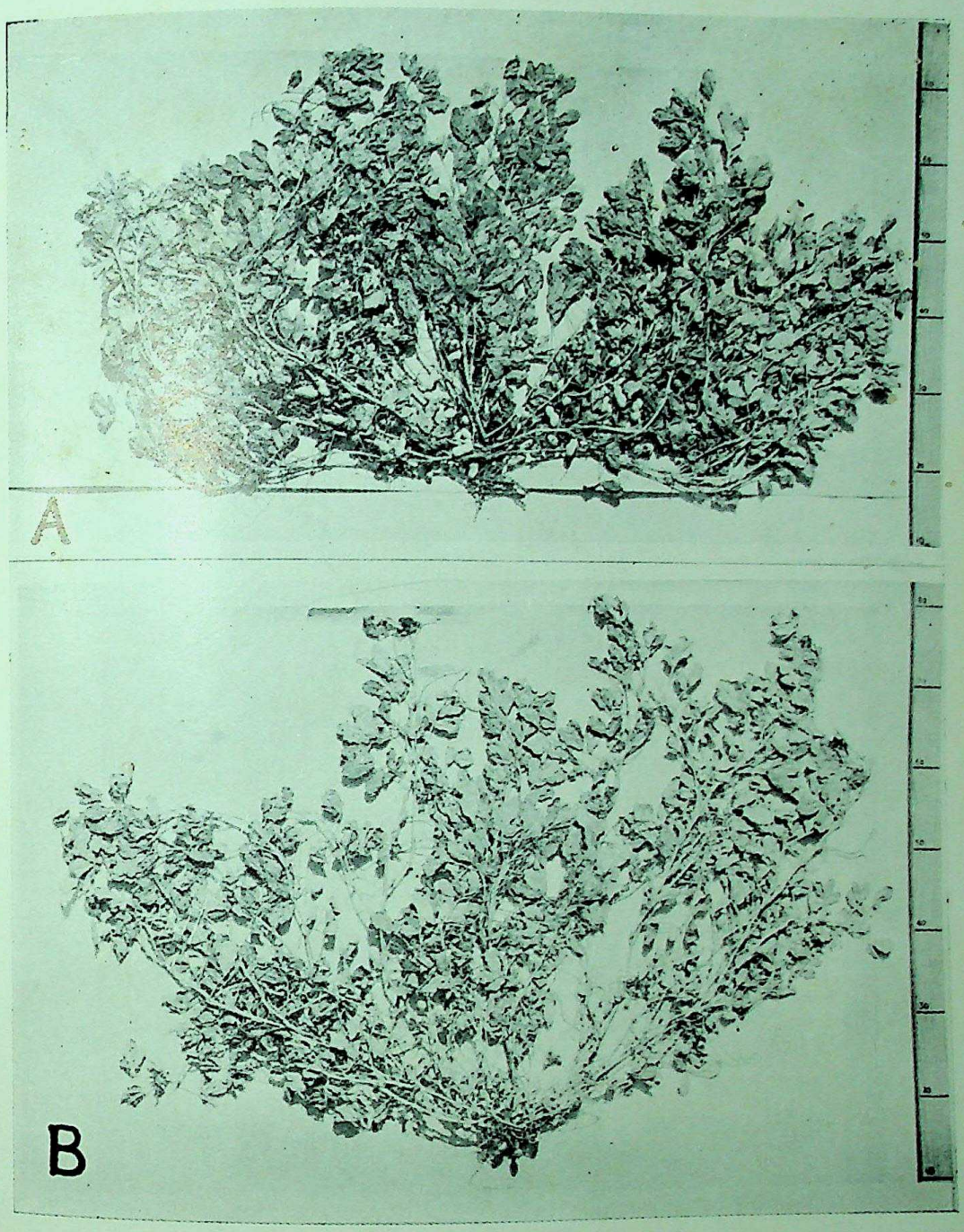
The regression coefficient i.e., the increase in yield loss per unit increase in disease intensity varied from 25.1 to 30.3 for pod weight and 24.9 to 30.3 for kernel weight in different years. The experimental results confirm the destructive nature of the disease resulting in total loss of yield of severely infected plants.

SUMMARY

Experiments were laid out in the field during 1958-63 to determine the disease-intensity-yield-loss relationships in respect of

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

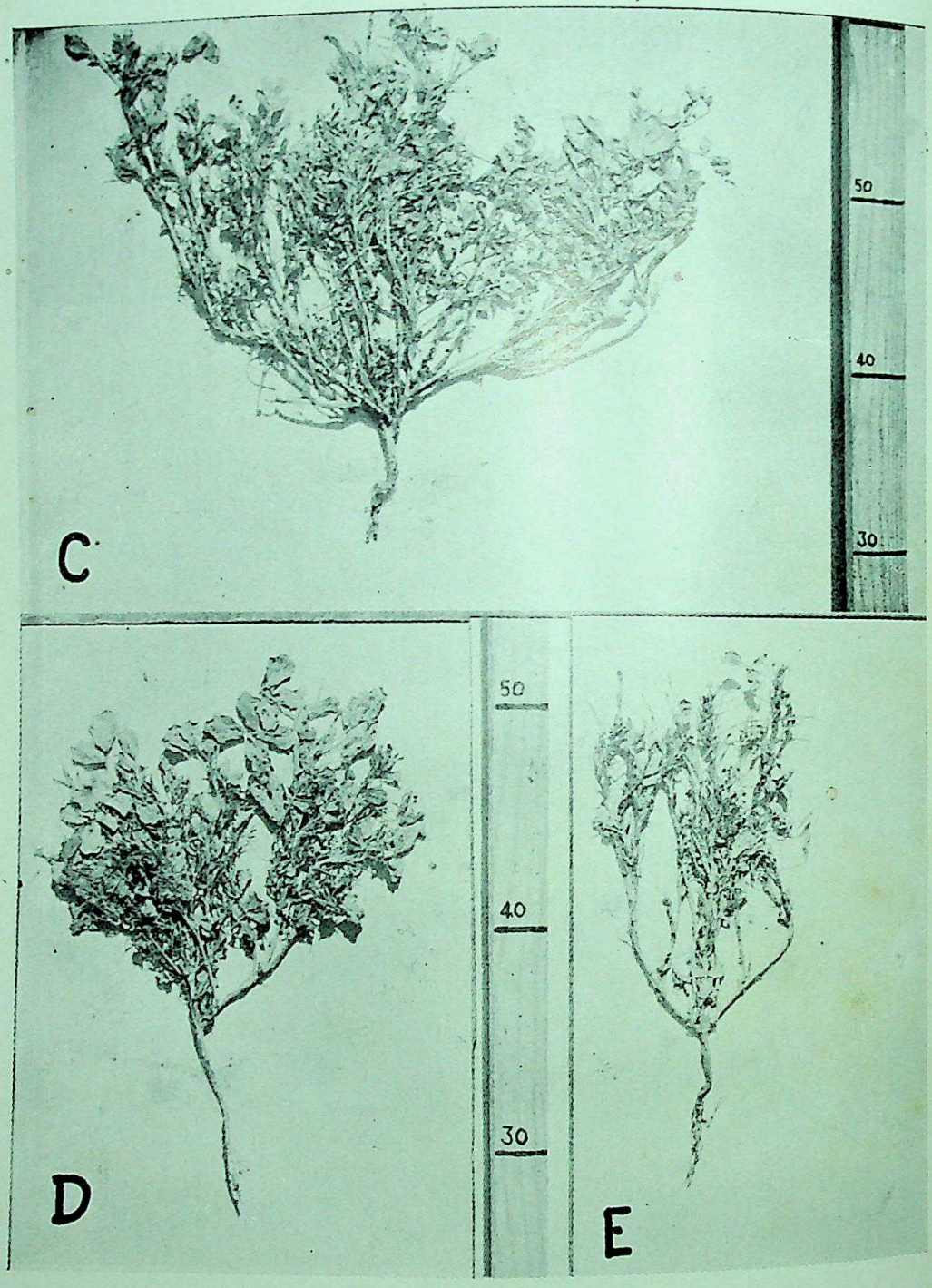
Digitized by Arya Samaj Foundation Chennai and eGangotri



Groundnut plants affected with mosaic of varying intensities
A—Healthy B—Light

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Groundnut plants affected with mosaic of varying intensities
C—Moderate D—Heavy E—Very heavy

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

mosaic disease of groundnut. A descriptive scale comprising of four grades of disease intensity was set up to grade disease intensity levels. The results of the experiments revealed that the loss in yield varied from 29-100 per cent in terms of kernel weight and 22-97 per cent in terms of pod weight depending upon disease intensity. The regression coefficient i.e., the increase in yield loss per unit increase in disease intensity varied from 25.1 to 30.3 per cent for pod weight and 24.9 to 30.3 per cent for kernel weight. The results thus point to the highly destructive nature of the disease.

ACKNOWLEDGEMENTS : The authors are thankful to Dr. B. L. Chona, and Dr. S.P. Raychaudhuri, the former and the present Head of the Division of Mycology and Plant Pathology, for providing necessary facilities and encouragement. Our thanks are due to Mr. Gorkha, Photographer, Indian Agricultural Research Institute, for taking the photographs.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute
New Delhi-12

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

GENUS CILIOCHORELLA IN INDIA

R. L. MUNJAL

(Accepted for publication February 19, 1966)

The genus *Ciliochorella* was established by Sydow (Sydow and Mitter, 1935) with *C. mangiferae* as the type species. Subramanian and Ramakrishnan (1953) described a new genus *Plagionema* and designated the type species as *P. indica*. The fungus was collected by them on a large number of hosts. Chona and Munjal (1955) after a comparison of the types of the two genera stated that they were congeneric and conspecific. Subramanian and Ramakrishnan (1956) working independently came to the same conclusion. Vishwanathan (1959) added a second species *C. eucalypti* and Kalani (1963) described a third species *C. indica*. A study of the type material of *C. indica* on *Cocos nucifera* (HC10.29048) and *Psidium guyava* (HC10.29049) revealed that the species was identical with *C. mangiferae* Syd. Despite efforts I have not been able to secure the type specimen of *C. eucalypti* Vishwanathan but the figures of spores given by the author, as also of *C. indica* and *C. eucalypti* by Kalani based on the type material leave no doubt that the latter is also conspecific with *C. mangiferae*. The discrepancies in the measurements of spores, as given by Sydow, Vishwanathan and Kalani are attributable to the fact that they have not included the thin ciliate part of the apical appendage in their measurements of the size of spores. The size of pycnidium, which was also taken into account by Vishwanathan is a variable character and so is the papillate or lenticular shape of the pycnidium, which was emphasized by Kalani as a differentiating feature. This leaves, only *C. mangiferae*, as the valid species of the genus.

A recent collection from Manali, Kulu district, Punjab (7000 ft.) on *Castanea europea* on examination showed that it belonged to the genus *Ciliochorella*, but being distinctly different in spore characters from *C. mangiferae* it is being proposed as a new species. Description of the fungus is given below :

The fungus forms minute, black, shining, erumpent pycnidia scattered on both surfaces of leaves, though more often on the lower surface. The pycnidia are subepidermal, dimidiate, upper half forming a shield, round, convex or plano-convex, measuring 600-850 μ in diameter, with a central papilla. The shield is brittle, opaque, 10-12 μ in thickness and is composed of 3-4 layers of dark-coloured prosenchymatous cells. The lower half of the pycnidium is composed of hyaline pseudoparenchymatous cells which form a stroma composed of 5-6 layers of cells in the middle but taper gradually into a single layer of cells towards the extremities extending beyond the shield. On the stroma is formed a closely packed layer of hyaline, conoid, straight conidiophores, measuring 6-8 μ in length and bearing conidia singly on their apices. The conidia are hyaline, oblong to concavo-convex, 15-18 x 3-4, μ, straight or slightly

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

curved, at times even sausage shaped. The upper end of the conidium is mostly rounded and blunt, lower end tapering, horizontally 3-septate, protoplasmic contents of the middle cells denser than those of the end cells; the upper cell is provided with a central apical seta that bifurcates to give antennae-like appearance, the lower cell is provided with a hyaline pointed seta attached to the concave side. The upper seta measures 33-36 μ in length and the lower one 5-8 μ in length. Some abnormal spores with 5-7 transverse septa with a single pointed apical and basal appendage were also observed.

The fungus grows readily on Oat-meal agar at 20-25°C., though it can withstand great variations of temperature from 5-35°C. It forms a felt-like mat on the surface of the medium, because the aerial mycelium is creeping. At first the colony is white but later becomes olivaceous. The black round scattered stromata measuring 1-3 mm in diameter are formed after about 15 days' growth, in which a large number of typical spores develop. The spores are formed on short phialophores.

Ciliochorella castaneae spec. nov.

Pycnidia nigra, dispersa, separata, raro bina simul, amphigena, innato-erumpentia, subepidermalia, dimidiata parte dimidia superiore efformante scutum, rotunda, 600-850 μ diameter, nitentia, convex vel plano-convexa una papilla cantrali ornata textibus scuti fragilibus, non translucida, 10-12 μ crassa, constantia e tribus ordinibus cellularum prosenchymaticarum; Pycnidiosporae hyalinae, 15-18 x 3-4 μ, rectae vel paulum curvatae, apice rotundato vel hebete, apice inferiore fastigato, 3-septatae, contentis protoplasmaticids cellulae mediae densioribus quam in cellulis apicalibus; cellula superna ornata una seta quae furcatur in quasi antennam aspectu; cellula inferior ornata una cellula hyalina fastigata. Seta superior 32-36 μ, inferior vero 5-8 μ longae. Sporophora brevia, recta, conica, 6-8 μ longa. (Fig. 1)

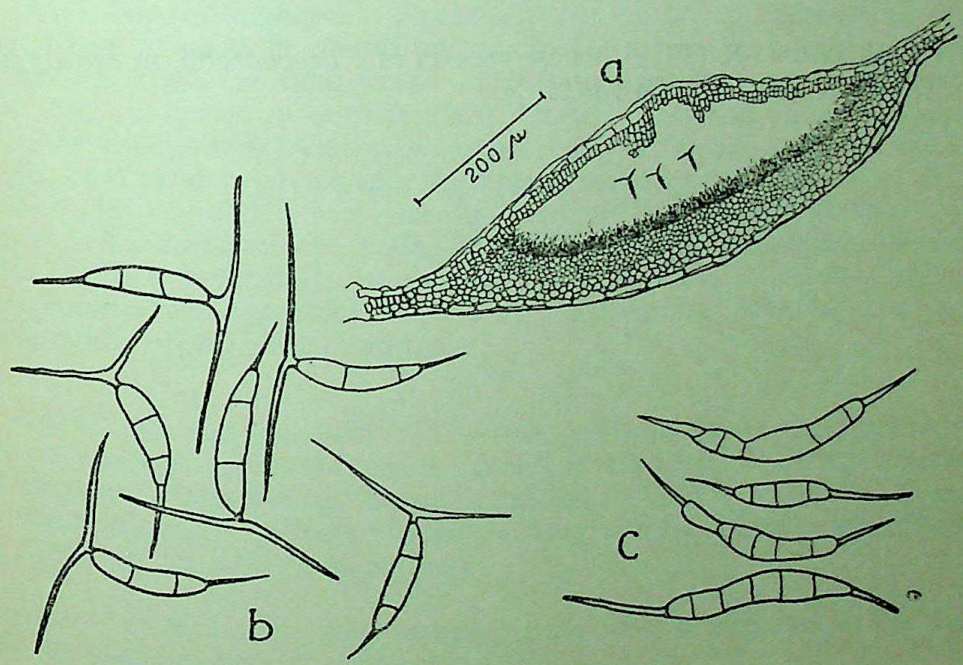


Fig. 1. *Ciliochorella castaneae* : (a) Pycnidium (b) Normal spores (c) Abnormal spores.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In follis solutis marcenscentibus *Castaneae europeae* ad Manali in Div. Kulu, in ditione Punjab, mense augusto anni 1962 leg. V.S. Sharma (Typus). H.C.I.O. No. 28239.

ACKNOWLEDGEMENTS : Sincere thanks are due to Dr. B.L. Chona, Head of the Division for encouragement and useful suggestions and to Prof. H. Santapau for very kindly translating the specific diagnosis into Latin. I am also indebted to Prof. M.N. Kamat for supplying type specimen of *Ciliochorella indica*.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute
New Delhi

REFERENCES

CHONA, B. L. AND R. L. MUNJAL (1965) *Plagionema* Subramanian & Ramakrishnan, a synonym of *Ciliochorella* Sydow. *Indian Phytopath* **8** : 64-75.

KALANI, I. (1963) Some new records of *Ciliochorella* spp. from India-Mycopath. et *Mycol. appl.* **19** : 238-240, 1963.

SUBRAMANIAN, C. V. AND K. RAMAKRISHNAN, (1953) *Plagionema*, a new genus of the Sphaeropsidales. J. *Indian Bot. Soc.* **32** : 131-136.

——————————AND ——————————(1956) *Ciliochorella* Sydow, Plagionema Subram. & RAMAKR. & Shanoria Gen. nov. Trans. *Brit. mycol. Soc.* **39** (3) : 314-318.

SYDOW, H. AND J. H. MITTER, (1935) Fungi indici II. *Ann. Mycol.* **33** : 46-71.

VISHWANATHAN, T. S. (1959) A new species of *Ciliochorella* on *Eucalyptus globulus*. *Curr. Sci.* **28** : 171.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CERTAIN PHYSIOLOGIC CHARACTERISTICS OF USTILAGO AVENAE AND U. KOLLERI*

AVDHESH NARAIN

(Accepted for Publication March 2, 1966)

INTRODUCTION : Loose and covered smuts of oats are caused by *Ustilago avenae* (Pers.) Rostr. and *U. kolleri* Wille respectively. These two species have been reported from all the oat-growing areas of the world. Both the smut species are facultative saprophytes and can be grown on culture media. In oat smuts it is recognized that the requirements and responses for growth in culture media vary somewhat, not only between two species but also within races of each species (Fisher and Holton, 1957). All smut fungi need some source of carbon, and nitrogen, as well as limited mineral nutrition. Certain sporidia-producing races of *U. avenae* and *U. kolleri* after subsequent transfers were made to produce mycelium by decreasing the amount of moisture (Rodenhiser, 1928). In the past many workers have studied the growth characteristics of oat and other smuts on a variety of culture media (Fischer and Holton, 1957; Rodenhiser, 1928 ; Das Gupta and Narain, 1960 ; Narain, 1962, 1964, and Sartoris, 1924).

The objective of the present study was to determine the effect of culture media and temperature on the rate of chlamydospore germination. Comparative growth on different solid and liquid media was also determined. Races, A14 and A22 of *U. avenae*, as well as collections, M5 and M11 of *U. kolleri* were used in this investigation. Recently, the collection M5 has been designated as race K9 (Luke, 1964). Since both these races of loose smut and collections of covered smut of oat have somewhat contrasting trends in their range of pathogenicity on many widely cultivated oat varieties in southeastern United States, it was considered logical to study their relative growth behaviour *in vitro*. Race A14 has a wide host range whereas race A22 has a narrow one (Luke *et al* 1961). Similarly, the collection M5 has a narrow host-range whereas M11 parasitizes a comparatively, wider host range. Such studies would reveal whether these 4 smut cultures, isolated on the basis of their inherent pathogenicity characteristics, exhibit any distinctive behaviour in their growth under controlled conditions.

MATERIAL AND METHODS : The smut material of A14 and A22 races of *U. avenae* was obtained from the late S.J. Hadden, Coker's Pedigreed Seed Company, Hartsville, South Carolina, U.S.A. Races, A14 and A22 were originally collected from Victorgrain (C.I. 3692) and Fulgrain (C.I. 3253) oat varieties respectively.

*Portion of Ph.D. thesis in Plant Pathology, submitted by the author at the University of Florida, Gainesville, Florida, U.S.A.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Blakeslee's agar, malt-extract agar, potato-dextrose agar, potato dextrose-malt-extract agar and water agar were used to test the rate of chlamydospore germination and other studies in culture.**

Chlamydospores were surface-sterilized by immersing in 0.1 per cent copper sulfate solution for 2-3 minutes, washed in sterile distilled water for 10 minutes and placed on the culture media. The rate of germination was determined by allowing spores to germinate on agar drops on slides. A drop of the respective melted agar medium was placed on a sterilized slide and allowed to solidify. A small drop from the spore suspension (250 spores/drop approximately) was transferred to the agar drop and the slide inside petri plate was incubated at 22-24°C. The percentage of spore germination was determined after 24, 48, 72, and 96 hours.

In the other experiment, the effect of 5 different temperatures and 4 culture media on spore germination was determined. A14 and A22 loose smut races as well as M5 and M11 covered smut collections were used in this study also. Spores were germinated on Blakeslee's agar, malt-extract agar, potato-dextrose agar, and potato-sucrose agar, at 20, 24, 28, 32, and 36°C. Respective smut cultures were placed inside the incubators which were set at appropriate temperatures and the data collected after 96 hours. Only the chlamydospores which produced well-developed promycelial cells were recorded at specified time-intervals in all the tests.

In addition, the comparative growth rates of the 4 smut cultures were determined on Blakeslee's agar, malt-extract agar, potato-dextrose agar, potato-dextrose-malt extract agar, and potato-sucrose agar. These tests were conducted in 9 cm petri plates, with 4 replications for each treatment. The inoculum was applied by transferring a 1 mm disc from vigrously growing sporidial cultures of each smut. The growth on solid media was estimated by measuring in millimeters the diameter of smut colony. All the cultures were allowed to grow for 3 weeks at 22-24°C. The average of 4 replications of each treatment was taken for record.

The comparative growth-rate of these cultures was determined on liquid media also by growing them in 250 ml Erlenmeyer flasks containing 35 ml of the above-mentioned liquids to which no agar was added. Three replications for each treatment were used. A1 mm disc of each sporidial culture (A14, A22 M5, and M11) was transferred to the flasks. Cultures were grown at 22-24°C and were constantly agitated over a shaker. The period allowed for growth was 3 weeks. The cultures were filtered through a Buchner funnel. Harvested fungal mats were oven-dried at 95°C for 12 hours and dry weights determined.

**(1) Blakeslees's agar:malt extract 20 g, dextrose 20 g, bacto peptone 1 g, bacto agar (Difco) 20 g, and distilled water 1 liter ; (2) Malt extract agar:malt extract 20 g, bacto peptone 1 g, bacto agar (Difco) 20 g, and distilled water 1 liter ; (3) Potato-dextrose agar:potato infusion from 200 g, dextrose 20 g, bacto agar (Difco) 20 g, and distilled water 1 liter ; (4) Potato-dextrose-malt-extract agar:potato-dextrose agar (Difco) 20 g, malt extract 20 g, and distilled water 1 liter ; (5) Water agar-bacto agar (Difco) 20 g, and distilled water 1 liter; (6) Potato-sucrose-agar:potato infusion from 200 g, sucrose 20 g, bacto agar (Difco) 20 g, and distilled water 1 liter.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RESULTS AND DISCUSSION : (a) *Effect of culture media on the chlamydospore germination* : Results (Fig. 1) indicate that after 96 hours the germination percentage between the loose smut races, A14 and A22 as compared to covered smut collections, M5 and M11 is highest on all the 5 culture media. The 2 races exhibit a material difference between the 72-and 96-hour test periods. This trend is not observed in the 2 covered smuts. In general, the loose smut races germinate more slowly on culture media than the covered smut collections.

Two contrasting trends are observed among the loose smut races. Race A14 germinates well (93 per cent) on Blakeslee's medium, while race A22 does not germinate at all or germinates in traces. On the other hand, race A22 expresses the highest rate (94 per cent) of germination on water agar, whereas race A14 has the lowest percentage (72 per cent) germination on this medium. No differential germination response is observed for the covered smut collections. M5 germinates equally well on all the five media ; however, the collection M11 expresses a differential germination response to the 5 media. M11 germinates best (72 per cent) on potato-dextrose agar and least (54 per cent) on water agar.

Chlamydospore germination tests reveal that in loose smut races A14 and A22, and in the collections M5 and M11 of covered smut spores germinate after 96 hours. The type of culture medium does not interfere with an increase in spore germination percentage along with the advance in time. Had this is not been the case, some irregularity would have been expressed in the pattern of chlamydospore germination. This may reflect that the highest stimulation in terms of moisture absorption and nutrition for chlamydospore germination is provided after 96 hours among the 4 smut cultures of *U. avenae* and *U. kolleri*. It is possible that to a limited extent the normal rate of chlamydospore germination is somewhat reduced after their surface sterilization with copper sulfate.

(b) *Effect of temperature and nutrient media on the chlamydospore germination* : Results (Fig. 2) show that the highest percentage of spore germination in the 4 smut cultures on all the 4 media viz. Blakeslee's agar, potato-dextrose agar, malt-extract agar, and potato-sucrose agar is attained at 22-32°C. In general, 20 and 36°C retard germination of all the cultures on all media. Hence, the range of 24-32°C can be rated as the optimum for loose smut races A14 and A22 as well as for the collections, M5 and M11 of covered smut. No marked interaction between the temperature and media is observed. A 14 and A 22 races of *U. avenae* germinate best (100 per cent and 83 per cent, respectively) on potato-dextrose agar at 28°C whereas M 5 and M11 collections of *U. kolleri* exhibit the highest percentage of chlamydospore germination (91 per cent and 75 per cent respectively) on this medium at 32°C.

(c) *Rate of growth on the solid media* : The results of this study (Fig. 3) indicate that races A 14 and A 22 of *U. avenae* and collections M 5 of *U. kolleri* grow vigorously on potato-dextrose-malt-extract agar (7.1, 6.7, and 6.4 mm respectively). The collection M 11 of covered smut is apparently a slow-growing organism. It exhibits the highest

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

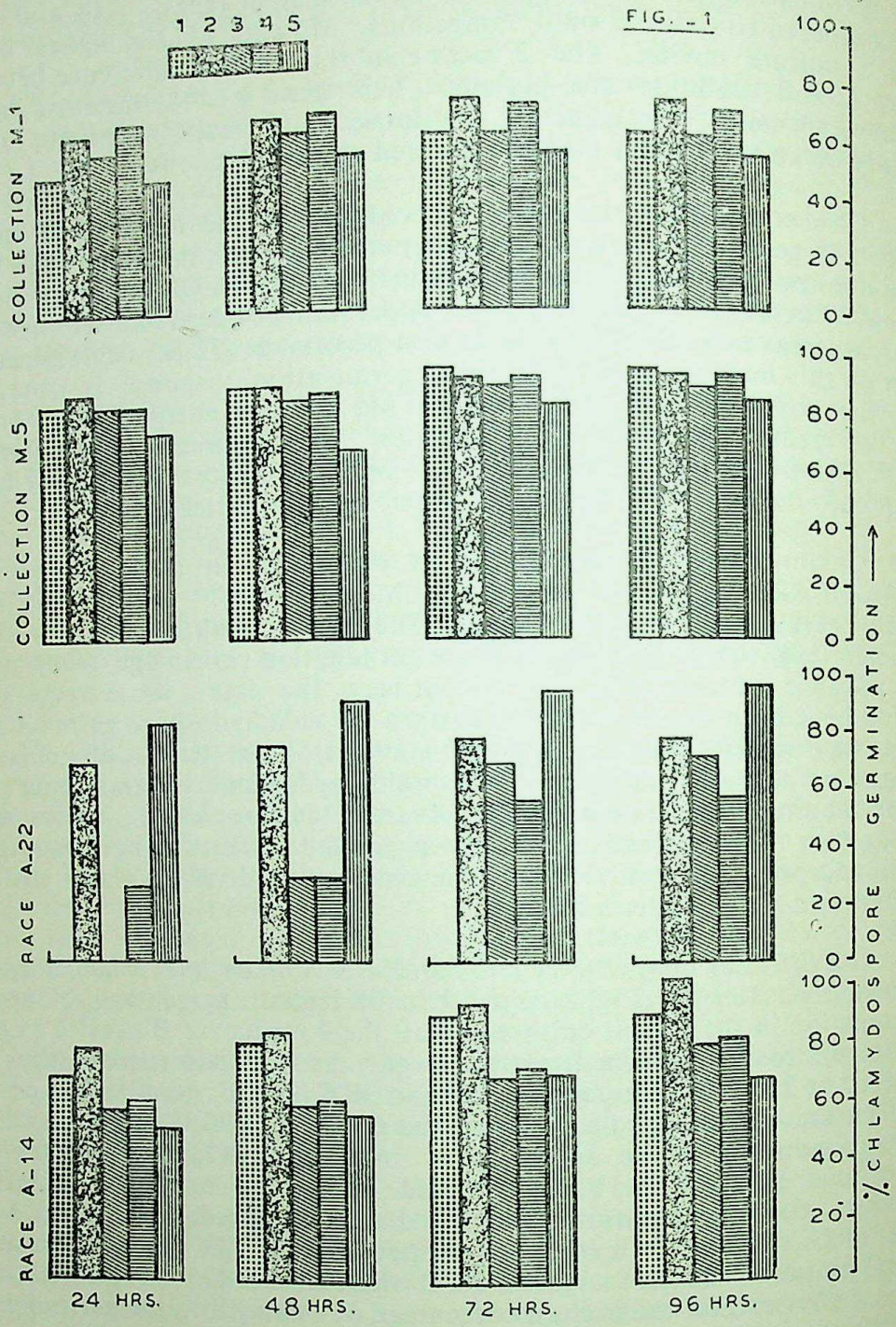


Fig. 1. Percentage of chlamydospore germination of races, A 14 and A 22 of *Ustilago avenae* and collections, M 5 and M 11 of *U kolleri* at various time intervals on selected culture media (1) Blakeslee's agar, (2) Potato dextrose agar, (3) Malt extract agar, (4) Potato dextrose-malt extract agar, (5) Water agar.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

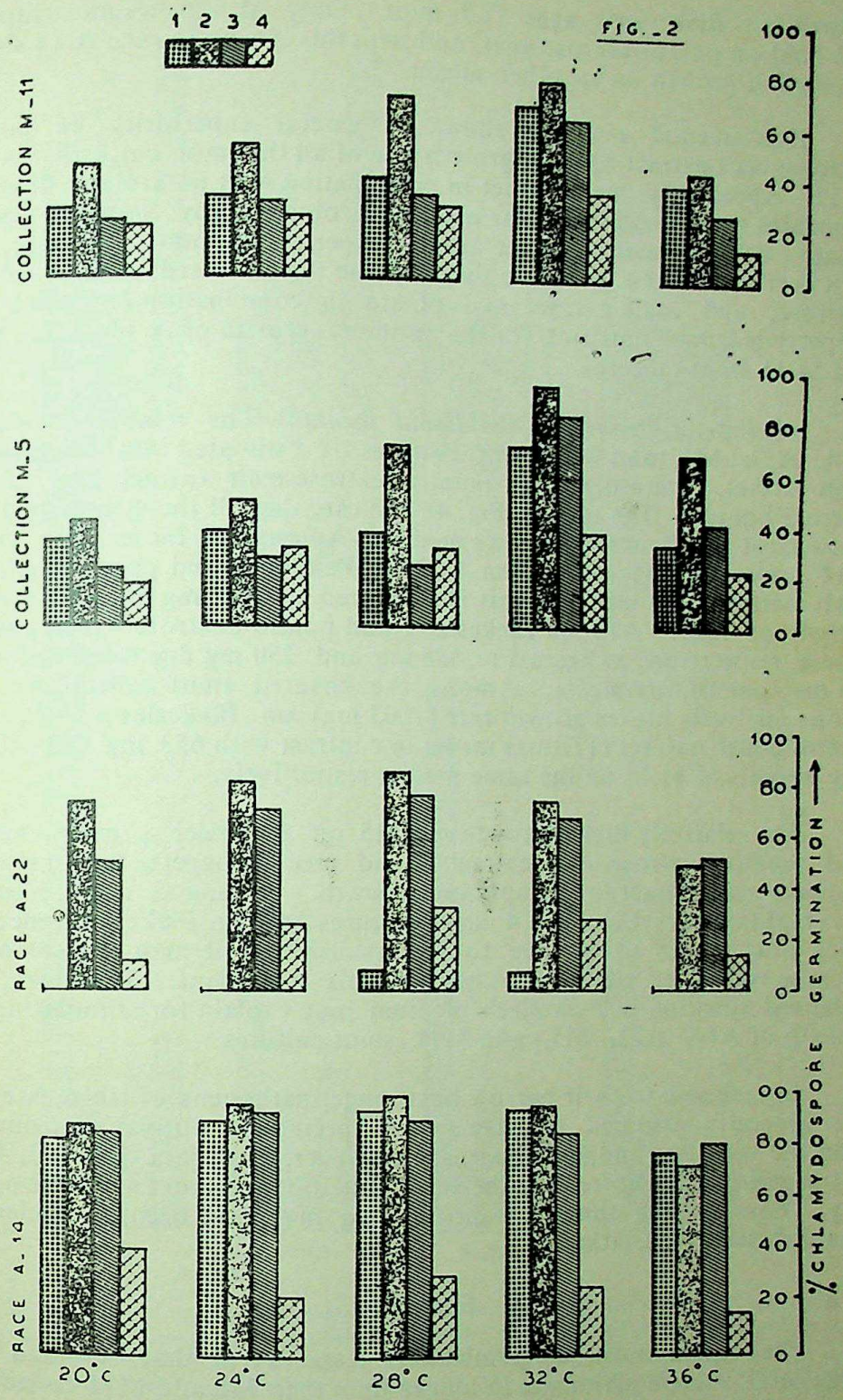


Fig. 2. Percentage of chlamydospore germination of races, A-14 and A-22 of *U. avenae* and collections, M-5 and M-11 of *U. kolleri* grown on 4 culture media for 96 hours, at various temperatures. (1) Blackeslee's agar, (2) Potato-dextrose agar, (3) Malt-extract agar, (4) Potato-sucrose agar.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

growth on Blakeslee's agar (2.7 mm). Only M 5 grows most rapidly (3.2 mm) on potato-sucrose agar, and with this exception race A 14 shows the highest growth on all other media.

Experimental evidence shows a general superiority of potato dextrose-malt extract agar for growth rate of all the smut cultures, except M 11. Apparently, malt extract in combination with potato and dextrose stimulates the growth. Similar results are obtained by Sartoris (1924). Potato dextrose-malt extract agar is superior to potato, dextrose, and malt extract, when used singly. Of the media used in this study, dextrose, and malt extract and potato in combination supplies the properly balanced nutrition for the optimum growth of A 14, A 22, M 5, and M 11 smut cultures.

(d) *Rate of growth on the liquid media* : The relative growth of A14, A22, M5, and M11 smut cultures is estimated on Blakeslee's, malt extract, potato dextrose, potato dextrose-malt extract and potato sucrose liquids. The results (Fig. 4) indicate that all the 4 smut cultures grow most rapidly on Blakeslee's medium. Among the loose smut races, A14 grows more vigrously than A 22 on Blakeslee's and potato dextrose-malt extract liquid media. This is evidenced by 1302 mg and 348 mg dry weight of races A14 on Blakeslee's and potato dextrose—malt extract media, respectively, as against to 436 mg and 250 mg dry weight of A 22 on the same culture media. Among the covered smut collections, M 5 shows relatively higher growth rate (1003 mg) on Blakeslee's and potato dextrose-malt extract (177 mg) media in contrast with 653 mg and 160 mg dry weights of M 11 on the same media, respectively.

The relatively higher growth of M5 on Blakeslee's, malt extract, and potato dextrose-malt extract liquid media suggests that this smut requires malt extract for its optimum growth. Among 5 culture media the highest growth of all 4 smut cultures only on Blakeslee's medium does not appear to be due to the availability of malt extract alone because two other media also contain this ingredient. Therefore, the balanced nutrition in Blakeslee's medium may explain for stimulating the growth of A14, A22, M5, and M11 smut cultures.

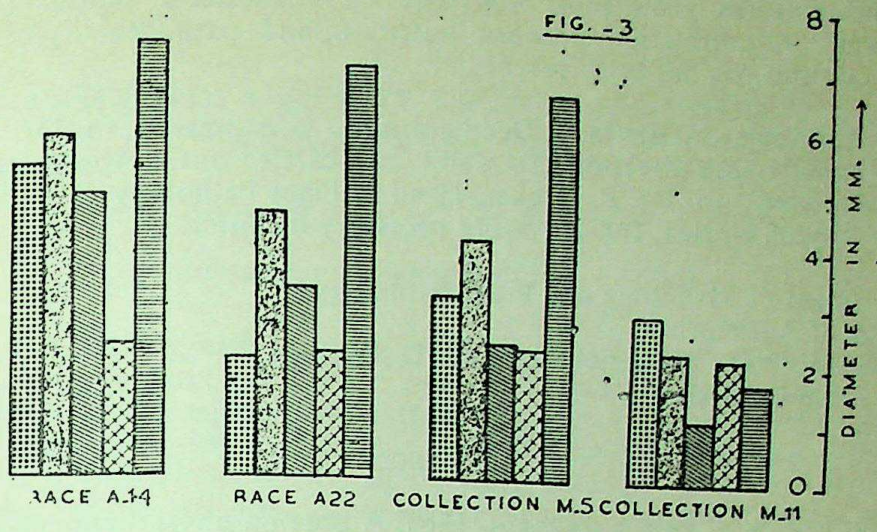
In response to their narrow host range, pathogens of this category are generally assumed to have a more specific nutritional environment than the wide-host-range pathogens. However, the data (Figs. 3, 4) do not suggest such a correlation between nutritional factors and pathogenicity. Possibly the range of media used may have been insufficient to establish such correlation.

SUMMARY

The chlamydospore germination tests reveal that A14 and A22 races of *U. avenae* germinate in longer time than M5 and M11 collections of *U. kolleri* on the agar media. The optimum temperature range for germination in 24-32 C. An interaction between the culture media and temperature has not been found. Among the solid media, potato dextrose-malt extract agar shows the best growth of A14, A22 and M5. All the

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



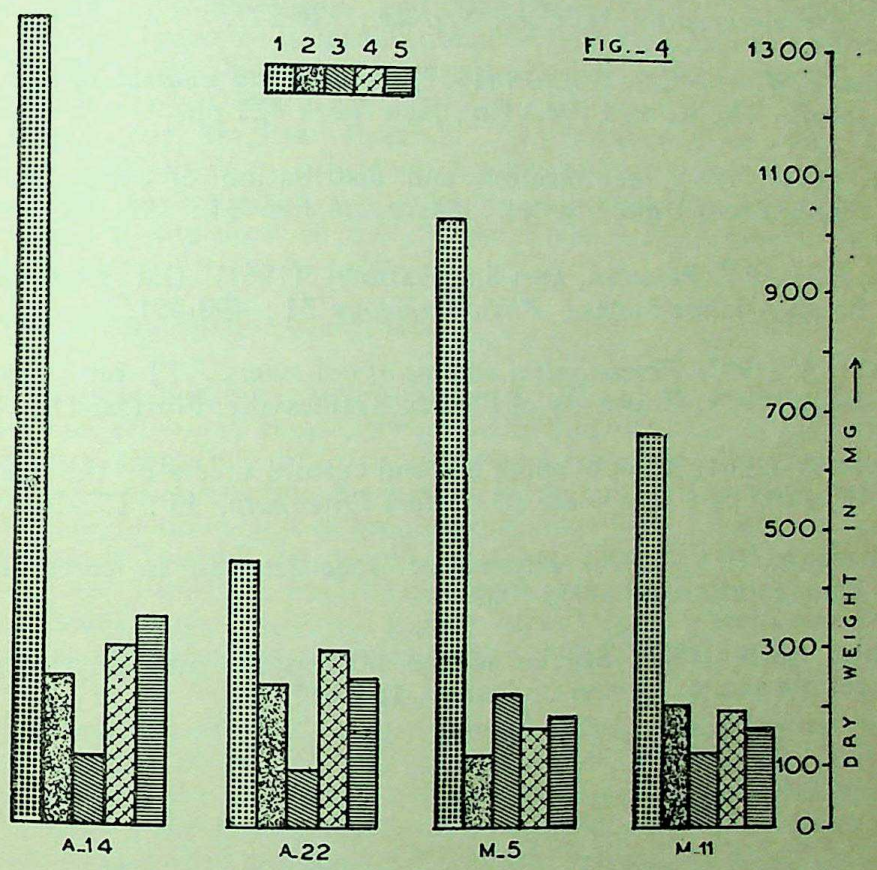


Fig. 3. Rate of growth (diameter measurements in millimeters) of races, A-14 and A-22 of *U. Avenae* and collections, M-5 and M-11 of *U. kolleri* on 5 culture media for a three week period. (1) Blakeslee's agar, (2) Potato dextrose agar, (3) Malt extract agar, (4) Potato sucrose agar, (5) Potato dextrose-nalt extract agar.

Fig. 4. Rate of growth (dry weights in milligrams) of races A-14 and A-22 of *U. avanae* and collections, M-5 and M-11 of *U. kolleri* on 5 liquid (shake) media for a three week period. (1) Blakeslee's medium, (2) Potato dextrose medium, (3) Malt extract medium, 4-Potato sucrose medium, 5-Potato dextrose-malt extract medium.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

4 smut cultures grow most vigrously on Blakeslee's liquid (shake) medium. A correlation between nutrition and pathogenicity range of smut cultures has not been revealed.

ACKNOWLEDGEMENTS : Deep gratitude is expressed to Dr. H.H. Luke under whose direction this study was carried out. Appreciation is also expressed to Dr. P. Decker, Head of Plant Pathology Department, University of Florida, for providing necessary facilities.

Department of Mycology and Plant Pathology
College of Agriculture
Orissa University of Agriculture and Technology
Bhubaneswar, Orissa

REFERENCES

DAS GUPTA, S.N. AND A. NARAIN (1960) A note on physiologic races of *Sphacelotheca sorghi*. *Curr. Sci.* **29** : 226-227.

FISCHER G.W. AND C.S. HOLTON (1957) *Biology and control of the smut fungi*. The Ronald Press Co., New York 622 pp.

LUKE, H.H. (1964) Identification and distribution of oat smuts of the Southeastern United States. *Phytopathology* **54** : 792-794.

LUKE, H.H., P.F. PFAHLER, AND S.J. HADDEN (1961) Oat smut races of South Atlantic States. *Phytopathology* **51** : 490-491.

NARAIN, A. (1962) *Physiological studies of oat smuts*. 72 pp. Doctoral Dissertation, University of Florida, Gainesville, Florida, U.S.A.

NARAIN, A. (1964) Effect of spore age and culture media on the degree of infection by *Ustilago avenae*. *Indian Phytopath.* **17** : 157-161.

RODENHISER, H.A. (1928) Physiologic specialization in cereal smuts. *Phytopathology* **18** : 955-1003.

SARTORIS, G.B. (1924) Studies in the life history and physiology of certain smuts. *Amer. J. Botany*, **11** : 617-647.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CERCOSPORA SPECIES CAUSING LEAFSPOT OF GRAPES IN INDIA

R. L. MUNJAL AND K. K. SETHI

(Accepted for publication March 4, 1966)

A leafspot of grapes caused by *Cercospora* species is of common occurrence throughout the world. Among the various species known to infect grapes, *Cercospora viticola* (Ces.) Sacc. is the most prevalent one, and is responsible for considerable economic losses. In India, there appear to be two species of *Cercospora*, which cause the leafspot of grapes. *C. viticola* has already been recorded by several workers (Sydow and McRae, 1929; Ramakrishnan and Sundaram, 1955; and Sethi and Munjal, 1963). The same fungus has been reported by Chona, Lall and Kakria (1958) from Delhi under the name *Pseudocercospora vitis* (Lev.) Speg., and by Hasija (1964) from Jabalpur, who have stated it to be a new record for Madhya Pradesh. In literature, we also find *C. vitis* (Lev.) Sacc. and *C. vitis* (Lev.) Lindau being commonly used for this organism. In this paper, it is proposed to discuss the taxonomy of *C. viticola* and also to describe *Cercospora riachueli* Speg., which is being recorded on grapes for the first time.

Cercospora viticola (Ces.) Sacc.

Leafspots circular, rarely irregular, 5 - 10 mm in diameter, dark-brown with an ashy-grey centre; fruiting-amphigenous, mostly hypophyllous; stroma present, but small; conidiophores fasciculate; fascicles dense, also loose but sometimes forming a compact coremium-like mass, olive-green changing to olive-brown, septate, geniculate with prominent scars, fairly uniform in breadth, but length variable, 3—6 x 50—400 μ. Conidia pale-olivaceous to medium dark-olivaceous-brown, multiseptate, obclavate, rarely with thick walls straight to slightly curved, base obconically truncate, blunt at the apex, usually attenuated, 4-7 x 20-80 μ (Fig. 1)

Perithecia scattered, mostly hypophyllous, subepidermal, later erumpent, globose, 60-91 x 60-89 μ; asci fasciculate, aparaphysate, clavate to elliptic. short stipitate, 28-41 x 6-9.5 μ, eight-spored, ascocpores irregularly biseriate, elliptic to broad-fusoid, both ends obtuse, bicelled, lower cell somewhat tapering, not constricted at the septum, 11-22 x 2.5-3.5 μ, hyaline. Spermagonia mostly hypophyllous, scattered, at first embedded, later erumpent, ovate to globose, 30-60 x 42-60 μ; spermatia small, rod-shaped, 2-3 x 1 μ, hyaline. (Fig. 2).

The above description agrees with the characters noted by various workers for *Cercospora viticola* and its perfect stage, *Mycosphaerella personata*, described by Higgins.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

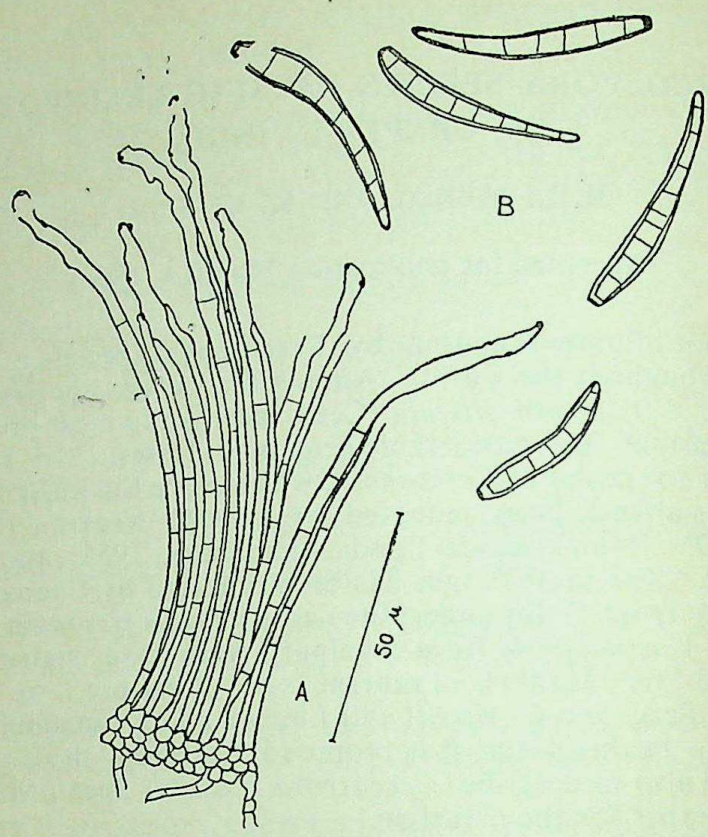


Fig. 1. *Cercospora viticola.* (A) Stroma with conidiophores. (B) Conidia.

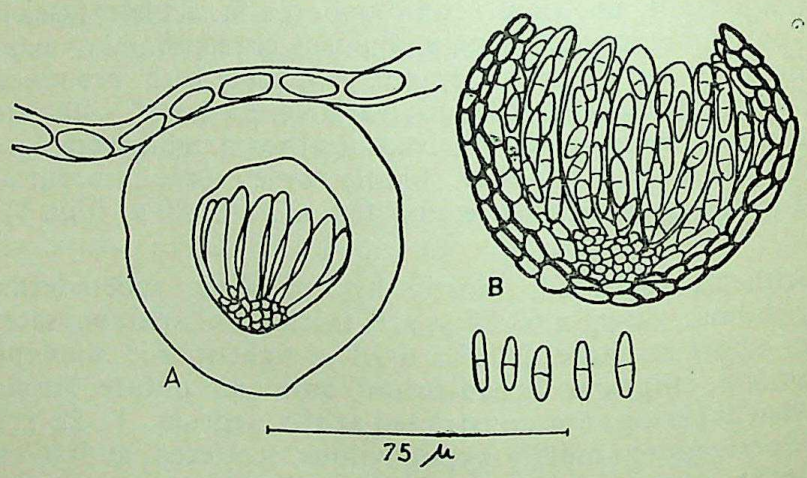


Fig. 2. *Mycosphaerella personata.* (A) Immature perithecium showing a bunch of asci. (B) Mature perithecium with ascospores.

This is the common experience of most workers on *Cercospora* that the size of conidia and conidiophores is considerably influenced by various environmental conditions, especially humidity. Welles (1925) experimentally

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

induced variation in the length of fruiting structures of several *Cercospora* species by varying the humidity. An experiment conducted on similar lines showed that conidia and conidiophores of *C. viticola* under natural dry field conditions measured 4—7 × 20—80 μ and 3—4 × 50—150 μ respectively, and the same leaves, when incubated in the laboratory in humid chamber (100 percent humidity), produced conidia and conidiophores measuring 4—7 × 60—100 μ and 4 × 100—600 μ respectively. Thus, there was considerable elongation of conidiophores under humid conditions.

Taxonomy of the fungus : Leveille (1848) appears to be the first person to have described a leafspot of grapes from Bordeaux, France, and named the causal fungus as *Septonema vitis*. The conidia are reported to be acrogenus, catenulate, subfusiform, 4 to 6 septate and deciduous.

In 1854, Cesati studying a similar disease in Italy, named the fungus as *Cladosporium viticolum*, while Passerini (1872) described the same as *Cladosporium ampelinum* and Berkeley and Cooke (1874) as *Graphium clavisporum*. Saccardo in 1875, apparently unaware of the earlier records, named this as *Cladosporium vitis*, which he next year transferred to *Cercospora vitis* and published the illustration of the fungus under this name in Fungi italici t671. In 1886, Saccardo when compiling the materials for his Sylloge came to know of the other names that had been applied to the same fungus. Following the principle of priority and nomenclatural rules, he named the same as *Cercospora viticola* (Ces.) Sacc. and listed all the above epithets as synonyms except *Septonema vitis* and remarked "vix. *Septonema vitis* Lev". He also changed *Graphium clavisporum* to *Isariopsis clavispora* but added the remarks "An forma compactior *Cercosporae viticolae* ?"

Other names, which have been misapplied to the same organism by some later workers are *Helminthosporium vitis* (Sacc.) Pirotta, *Cercospora vitis* var. *rupestris* Ciferri and *Pseudocercospora vitis* (Lev.) Speg.

Rhoads (1926) identified this organism as *Isariopsis clavispora* Sacc. The illustration given by him shows the conidiophores forming synnemata and spores attached to the conidiophores at the acute ends. It is now well recognised by the workers dealing with this fungus that under dry conditions, the conidiophores are free but under humid conditions they become elongated and are agglutinised in the form of bundles, so as to appear as synnemata. The attachment of spores, as shown by Rhoads is a gross mistake. The conidia have been reported to be deciduous by various workers. It appears Rhoads saw those conidia, which had fallen from the conidiophores and got stuck upside down.

Spegazzini (1910) transferred this fungus to his new genus *Pseudocercospora* and characterised it as *Cercospora* species with dark, pluriseptate, catenulate conidia and distinctly long conidiophores. The elongation of conidiophores is dependant on environmental conditions, chiefly humidity, as has already been experimentally proved. In the numerous collections examined by us, we have never come across catenulate conidia on the natural substrate. Therefore the criteria laid down for the erection of the genus *Pseudocercospora* are not tenable, at least in so far as *C.viticola* is concerned. Moreover, the prefect stage of *C. viticola* observed by

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Higgins and ourselves is a *Mycosphaerella*, which shows that this fungus has more affinity with *Cercospora*.

In the current literature, particularly the abstracting journals like Review of Applied Mycology, Biological abstracts, etc., two epithets, namely, *C. vitis* and *C. viticola* appear very frequently and they refer to the same fungus. This confusion has probably crept in with the publication of Rabenhorst's kryptogamen Flora Vol. IX, 1910, in which Lindau recognising *Septonema vitis* (Lev.) as basonym made a new combination *Cercospora vitis* (Lev.) Lindau. This nomenclatural change is not tenable, because it is a later homonym of *C. vitis* (Lev.) Sacc. (1877), which has been already rejected by Saccardo himself. Thus, *Cercospora viticola* (Ces.) Sacc. is the only valid name for the imperfect stage of this fungus.

Cercospora viticola (Ces.) Sacc.

=*Mycosphaerella personata* Higgins

Syn.

1854 *Cladosporium viticolum* Cesati. lora **38** : 206.
1872 *Cladosporium ampelinum* Pass. Erb. Critt. Ital. n. 595.
1874 *Graphium clavisporum* Berk. and Cke. Grev. **3** : 100.
1875 *Cladosporium vitis* Sacc. Myc. venet. n. 284.
1876 *Cercospora vitis* (Lev.) Sacc. Nuov. Giorn. Bot. Ital. **8** : 188.
1886 ,, *viticola* (Ces.) Sacc. Syll. ung. **4** : 458.
1886 *Isariopsis clavispora* (B. and C.) Sacc. Syll. Fung. **4** : 631.
1889 *Helminthosporium vitis* (Sacc.) Pirotta, Rev. Mycol. **11** : 185
1910 *Pseudocercospora vitis* (Lev.) Speg. Anal. Mus. Nac. B. Aires. **20** : 437
1910 *Cercospora vitis* (Lev.) Lindau, Rabenh. Krypt. flora, **9** : 116.
1922 *Cercospora vitis* var. *rupsetris* Cifferri, Ann. Mycol. **20** : 45.

Cercospora riachueli Speg.

in Anal. Soc. Cient. Argentina **10** : 38, 1880

Leafspots irregular, 2—8 mm. in diameter, dirty-brown to tan-coloured, fruiting hypophyllous, stroma present, subglobose, dilutely coloured, 15—80 μ in diameter: fascicles dense, forming a compact layer, conidiophores dilutely coloured, short, unbranched, sparingly septate, geniculations not distinct, irregular in width, 3—4 × 10—15 μ; Conidia obclavate-cylindric, lightly coloured, curved, multi-septate, base obconically truncate, tip subobtuse, 3.5—4.5 × 25—80 μ. (Fig. 3).

On living leaves of *Vitis vinifera* L., Kulu (Punjab) V. S. Sharma, July 10, 1958, HC10 No. 26612 and on V. *trifolia*, Ridge Road, New Delhi, R. L. Munjal October 17, 1955 HCIO No. 25957.

The characters of this fungus agree with *C. riachueli* speg., as given by Saccardo in Sylloge Fungorum Vol. IV p. 458, 1886 and the description of this fungus given by Chupp (1953) in his monograph on Cercospora. It has been recorded so far on *Cissus* sp. The fungus is rare in its distribution, as it has been collected only once in India on cultivated and wild grape.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

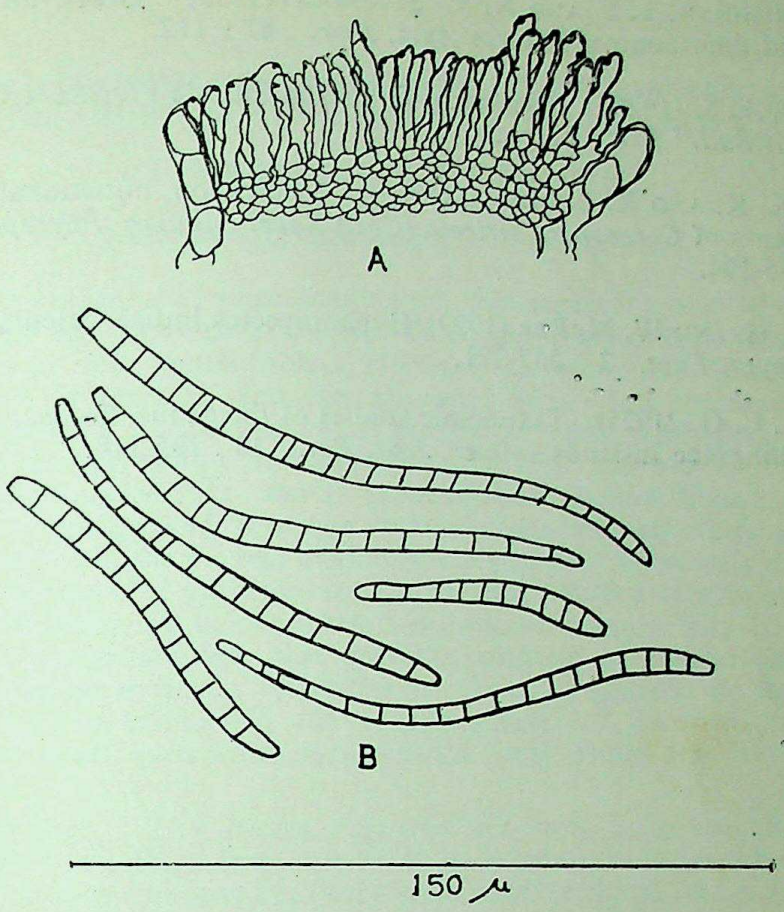


Fig. 3. *Cercospora riachueli* (A) Stroma with conidiophores. (B) Conidia.

Thanks are due to Dr. R. S. Vasudeva, former Head of the Division of Mycology and Plant Pathology for providing necessary facilities for this work.

Division of Mycology and Plant Pathology
Indian Agricultural Research Institute
New Delhi

References

CHONA, B. L., G. LALL, AND N. C. KAKRIA (1958) The Fungi of Delhi *ICAR. Bull.* No. 81, p. 29.

CHUPP, C. (1953) A Monograph of the fungus genus *Cercospora* Ithaca, New York 667 pp.

HASIJA, S. K. (1964) Additions to the fungi of Jabalpur (Madhya Pradesh) -IV-*Mycopath. Mycol.* **23** ; 235-240.

HIGGINS, B. B. (1929) Morphology and Life-history of some Ascomycetes with special reference to the presence and function of spermatia II-*Amer. Jour. Bot.* **16** : 287-296.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RAMAKRISHNAN, T. S. AND N. V. SUNDRAM (1955) Grapevine diseases and their control-Madras *Agri. Jour.* **42** : 112.

RHOADS, A. S. (1926) Diseases of Grapes in Florida-Florida *Agri. Exper. Sat.Bull.* **178** : 82 pp.

SETHI, K. K. AND R. L. Munjal (1963) Studies on nutritional requirements of *Cercospora viticola* (*Ces.*) *Sacc.* *Indian Phytopath.* **16** : 185-194.

SYDOW, H. AND W. McRae (1929) Hyphomycetes Indiae Orientalis—*Ann. Crypt. Exot.* **2** : 262-271.

WELLES, C. G. (1925) Taxonomic studies of the genus *Cercospora* in the Philippine Inslands—*Amer. Jour. Bot.* **12** : 195-218.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

SOME AEROBIOLOGICAL OBSERVATIONS ON THE RICE STACKBURN FUNGUS, TRICHOCONIS PADWICKII

T. SREERAMULU AND B.P.R. VITTAL

(Accepted for publication March 4, 1966)

Information on some aspects of the aerobiology of the fungus *Trichoconis* was provided by Panzer, Tullis and Arsdel (1957), Chandwani, Balakrishnan and Padmanabhan (1963), Sreeramulu and Seshavataram (1962) and Sreeramulu and Ramalingam (1963). In a continuous two-year study of the air-spora at Visakhapatnam, using a Hirst spor-trap, (Hirst, 1952), Sreeramulu and Ramalingam (1966) reported that spores of *Trichoconis padwickii* the causal agent of the stackburn disease of rice, exhibited a well-marked '*forenoon pattern*' of diurnal periodicity and that their incidence in the air was distinctly seasonal and was closely related to the growth phases of the rice crop in the field. With a view to providing more information on their interrelationships, air-sampling was conducted with a Hirst trap in a rice plot specially planted at Anakapalle. The relationships between the variations in the spore catches, the changes in the weather conditions and the growth phases of the rice plants (in the experimental plot which served as the local source) are discussed in this paper.

METHODS : The results reported are from an air-sampling study conducted with a Hirst trap located at m-1 height in the centre of a plot (transplanted on August 11, 1962) with rice seedlings of *Co. 13* variety surrounded by fields with *Mtu* 19 (transplanted on 3 and 4, August 1962) at the Sugarcane Research Station, Anakapalle, A.P. Air-sampling methods followed and mounting and scanning procedures adopted were in general the same as those described by Sreeramulu and Seshavataram (1962). Hyaline to creamy-yellow, elongately fusoid conidia, with a long appendage at the tip, 3to5-septate constricted at the septa, with the second or the third cell from the base larger than the rest, measuring 100-170 μ long (including the appendage) and 10-19 μ broad were identified as the conidia of *T. padwickii* and were counted as the spores of the stackburn fungus.

DIURNAL PERIODICITY : The diurnal-periodicity curve shown in Fig. 1 is constructed from the estimated percentages of the peak arithmetic mean concentration observed at different hours on several representative normal days in the period of this study in 1962. The diurnal rhythm observed in 1962 agrees very well with that reported earlier by Sreeramulu and Seshavataram (1962) and Sreeramulu and Ramalingam (1966), in showing typical '*forenoon pattern*' . The daily peak is observed recurring at 08.00 hr. in this study at Anakapalle. In the entire trapping period the highest hourly concentration observed was *c.* 2,270/m.^{3} at 08.00 hr. on the 28th October.

SEASONAL CHANGES : Although spore-trapping was started on the 11th August, the day on which *Co. 13* seedlings were transplanted, spores

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

of *T. padwickii* did not appear in the air till the 18th August. On this day two spores were caught at 06.00 hr., and subsequently on August 19, 21, 24 and 29 a few spores of this fungus were encountered in the forenoon hours. Variations in the estimated daily mean concentrations from 1 September to 13 December are shown in Fig. 2 along with changes in the weather conditions.

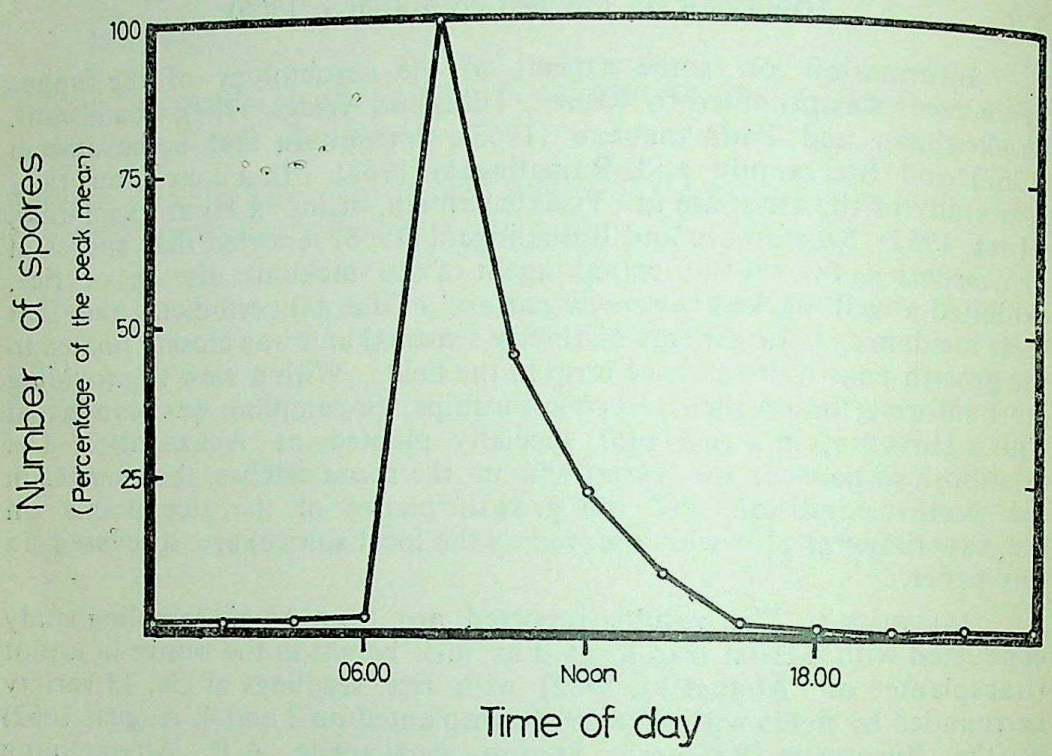


Fig. 1.. Diurnal-periodicity curve expressed as percentages of the peak arithmetic mean concentration.

Records on the course of growth of *Co. 13* rice plants taken according to the scale given by Matsuo (1957) indicated that by September 12 rice plants grew up to a height of 20 cm. and were passing through 'mid-tillering' phase. They grew to a height of 50-60 cm. by September 30 and were passing through the 'booting' and 'heading' phases. Till the end of September very few spores of the stackburn fungus appeared in the air. In the month of October a gradual increase in their numbers was seen in the first 3 weeks—the period when the rice plants in the experimental plot were passing through the 'heading' phases. Records from October 13to19 showed that the host plants grew to a height of 80-100 cm. and that many of them had completed the 'heading' phase and entered into the 'early ripening' phase. By 14th November the *Co. 13* crop was ripe and was ready for harvest. From the variations in the catches it can be said that the period of maximum occurrence of the spores of the stackburn fungus in the air coincides with the 'heading' and the 'ripening' phases of the crop. As the *Co. 13* crop was harvested on November 16 there was a sudden fall in the catches. The appreciable number of spores

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

encountered in air from November 21 to 29 were probably from the surrounding *Mtu. 19* crop which was then passing through the 'mid'-and 'late-ripening' phases. The catches in December were associated with the harvesting operations in the vicinity.

Thus, from the seasonal periodicity data it is clear that the stack-burn fungus is important as a grain-infecting organism and that its maximum spread occurs in the period when the heading of the crop is over, and when it enters into the 'ripening' phases.

EFFECTS OF WEATHER : Two-hourly changes in the concentrations of the airborne spores of *T. padwickii* are shown along with changes in the weather in Fig. 3 for the periods from October 17 to 24 and from October 30 to November 6 to show how changes in the existing weather affect their numbers and their diurnal rhythms.

Two-hourly changes in the period from 08.00 hr. on October 17 to 06.00 hr. on October 25 1962 (Fig. 3) : In this period till the night of Oct. 22 the conditions were dry and normal for this part of the year. Typical diurnal rhythm with the daily maxima recurring at 08.00 hr. was maintained on all these 6 days. On Oct. 23 it was cloudy and although there was a rainfall of 30.4 mm. spread over almost throughout the day, there was no rain from 06.00 to 11.00 hr. which are the peak hours for this type. It is interesting to find that during these hours appreciable numbers of spores appeared in the air and the normal diurnal rhythm was maintained on this day. On Oct. 24 spores of this fungus were almost completely absent from the air as there was a continuous rainfall of 83.7 mm. throughout the day. From the 24/25 midnight till 06.00 hr. on Oct. 25 there was a very heavy rainfall of 84.8 mm. and no spores of this type appeared in the air on this day.

Two-hourly changes in the period from 00.00 hr. on 30 October to 24.00 hr. on 6 November 1962 (Fig. 3) : The rainy period which started on the night of Oct. 22 continued till Nov. 2 with two or three clear days in between. During this wet period seasonal maxima in the incidence of the air-borne spore-loads of the stackburn fungus were noticed. The changes observed from Oct. 30 to Nov. 6 show how a gradual fall in their catches occurred after this rainy spell.

Typical diurnal periodicity patterns were maintained on all these days. Although there was rain in the nights of 31 Oct./1 Nov. and 1/2 Nov. regular diurnal rhythms were noticed even on these days.

Thus, from the data it can be said that rain in general has a major effect. The catches observed on Oct. 23 and Nov. 2 clearly indicate that the stackburn fungus maintains its diurnal rhythm if there is no rainfall at its peak hours even though this period is preceded by rain.

DISCUSSION : In India, stackburn of rice was first recorded by Padwick and Ganguly (1945) in the form of a disease of the grains in the ear-head. Ganguly (1947), in a detailed account of the disease, reported that only a few grains in an ear-head were generally infected. Tullis (1936) remarked that the fungus gained entry through the glumes and attacks

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

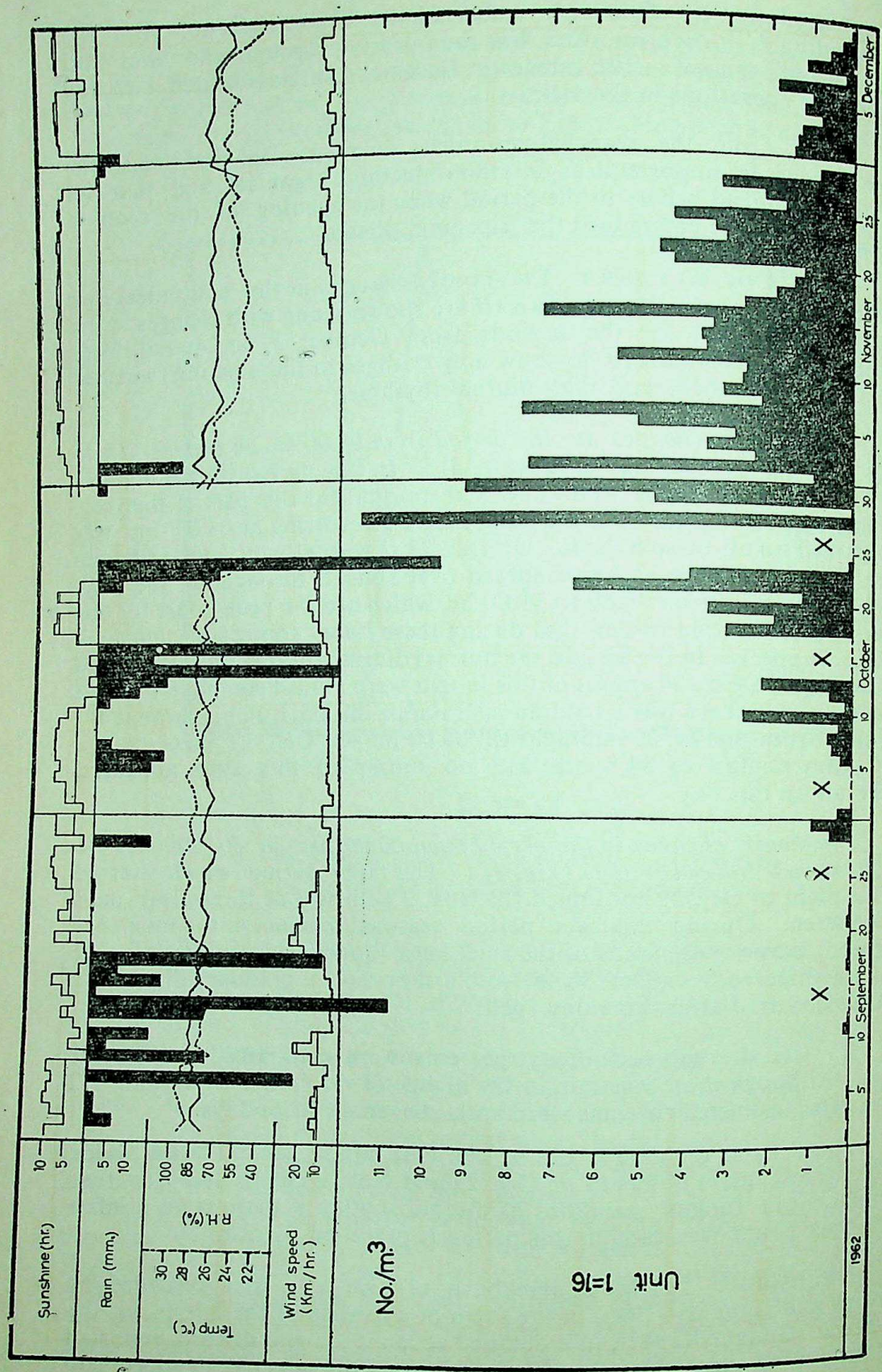


Fig. 2. Changes in the daily mean concentrations related to changes in the weather conditions ; the periods when the trap was not working are indicated by X.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

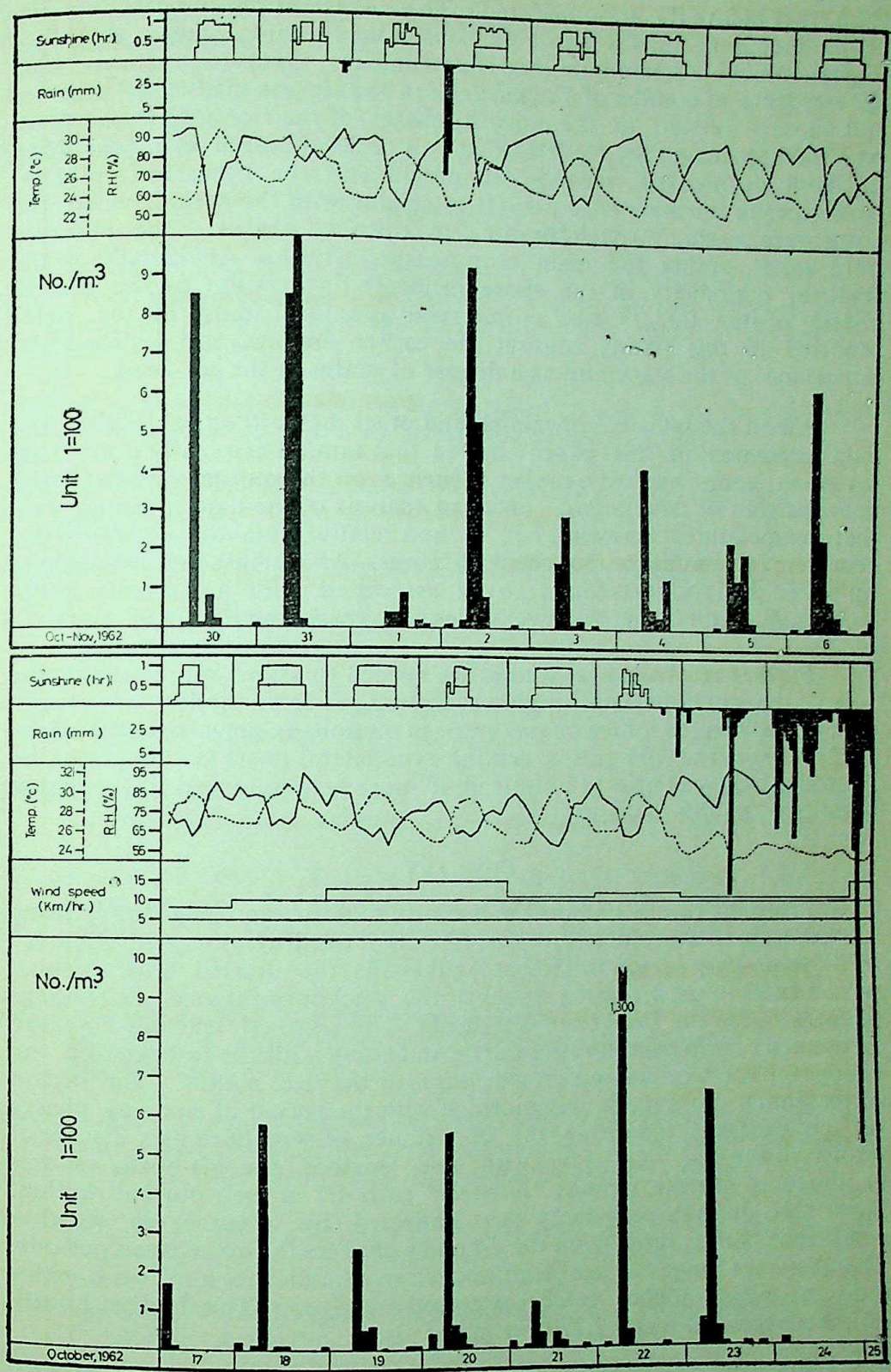


Fig. 3. Two-hourly estimates of the concentrations related to changes in weather conditions.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the kernel before the grain matured. These observations indicate that air-borne inoculum infects the host plant at the 'heading' stage. Aerobiological studies (Sreeramulu and Ramalingam, 1966) have shown that the incidence of conidia of *T. padwickii* in the air was distinctly seasonal and closely related to the growth phases of the rice crop in the field. At Visakhapatnam they reported that two peaks appeared in a year (one in April during the second-crop period and the other in October—November in the main-crop period) coinciding with the periods when the crops were passing through 'heading' to 'ripening' phases. The observations made during the main crop season in 1962 at Anakapalle on the seasonal periodicity in the spore incidence in relation to the growth phases of the *Co. 13* rice crop serving as a local source in the field, reported in this paper, confirm the earlier findings and indicate the importance of the stackburn as a disease of grains in the ear-head.

About the influence of rainfall and other meteorological factors the data presented in this paper showed that rainfall especially during the forenoon hours exerted a major influence on the diurnal rhythm in the spore catches of this fungus. From an analysis of the data it can be said that temperatures between 27-31°C. and relative humidities of 50-70 per cent were favourable for the spread of spores. An increase in wind velocity up to 10 Km./hr. was found to be associated with an increase in the catches, but with higher wind velocities a decrease was observed.

Further aerobiological studies are needed to know more about the role of the environmental and host factors. The possibility of annual recurrence from the source on rice straw in the soil (as suggested by Tisdale, 1922) or from the wild grasses serving as collateral hosts (as suggested by Padwick, 1950) can also be examined, if more aerobiological information is gathered on this desease.

Summary

From the results obtained in a continuous air-sampling study, using a Hirst trap, in a rice plot with *Co. 13* surrounded by plots with *Mtu. 19* in the main-crop season in 1962 at Anakapalle. the diurnal and seasonal periodicities in the air-borne spores of the stackburn fungus—*Trichoconis padwickii*—at 1m. height are described. The seasonal changes observed in the daily mean concentrations are given along with the information on changes in the weather and growth stages of the rice plants. The period of maximum spore incidence coincided with the period of ripening phases of the earheads indicating the importance of stackburn as a disease of grains in the ear. On days with fine weather the air-borne spores exhibited a sharply defined '*forenoon pattern*' in their diurnal rhythm. How this diurnal periodicity was affected by changes in weather conditions is illustrated from the 2-hourly changes in two selected periods. The optimum ranges of temperatures, relative humidities and wind velocities at which higher catches occurred are given. The highest hourly estimate recorded was *c* 2,270 per m.^{3} of air.

Acknowledgements: We are grateful to Messrs. M. Lakshmikantham, P. Prakasam and P. Appalanarasayya of the Sugarcane Research Station, Anakapalle, for the facilities and for help in various ways in

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

conducting this work. Acknowledgement is also made of the help received from Dr. A. Ramalingam.

Botany Department
Andhra University
Waltair, (A. P.)

REFERENCES

CHANDWANI, G. H., M. S. BALAKRISHNAN AND S. Y. PADMANABHAN (1963) *Helminthosporium* disease of rice V. A study of the spore population of *Helminthosporium oryzae* over rice fields. *J. Indian bot. Soc.* **42** : 1-14.

GANGULY, D (1947) Studies on the stackburn disease of rice and identity of the causal organism. *J. Indian bot. Soc.*26 : 233-239.

HIRST J. M. (1952) An automatic volumetric spore trap. *Ann. appl. Biol.* 39 : 257-265.

MATSUO, T. (1957) *Rice culture in Japan.* Tokyo : Yokendo, Ltd.

PADWICK, G. W. (1950) *Manual of rice diseases.* Kew : Commonwealth Mycological Institute.

PADWICK, G. W. AND D. GANGULY (1945) Stackburn disease of rice in Bengal. *Curr. Sci.* **14** : 328-329.

PANZER, J. D., E. C. TULLIS AND E. P. VAN ARSDEL (1957) A simple 24-hour slide spore collector. *Phytopathology* **47** : 512-514.

SREERAMULU, T. AND A. RAMALINGAM (1963) Spore content of air over paddy fields. II. Changes in a field near Visakhapatnam from November 3, 1959 to January 9, 1960. *Proc. nat. Acad. Sci. India,* **33 B** : 423-428.

SREERAMULU, T. AND A. RAMALINGAM (1966) A two-year study of the air-spora of a paddy field near Visakhapatnam. *Indian J. agric. Sci.* **36** : 105-126.

SREERAMULU, T. AND V. SESHAVATARAM (1962) Spore content of air over paddy fields. I. Changes in a field near Pentapadu from 21 September to 31 December, 1957. *Indian Phytopath.* **15** : 61-74.

*TISDALE, W. H. (1922) Seedling blight and stackburn of rice and the hot water seed treatment. *Bull U. S. Dep. Agric.* No. 116.

*TULLIS, E. C. (1936) Fungi isolated from discoloured rice kernels. *Bull. U. S. Dep. Agric* No. 540.

*Originals not seen.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

RESISTANCE OF PADDY VARIETIES AND CULTURES TO BLAST IN CHHOTA NAGPUR

A. K. DUTTA AND S. M. GHUFRAN

(Accepted for publication March 8, 1966)

Blast of paddy caused by *Piricularia oryzae* Cav. occurs in Chhota Nagpur both as a leaf-spot in the seedling and tillering stages and as neck infections at the time of flowering. It is prevalent in other regions of Bihar also and has been noticed to be on the increase during the recent years. During the last few years the testing of improved paddy varities and cultures for blast resistance has been a regular feature of blast-control work in Bihar.

MATERIAL AND METHODS : The experiments were conducted at Netarhat, situated at an altitude of 3,700 feet above sea-level, where the incidence of blast has been found to be heavy on the susceptible varieties.

The seedlings were sown in replicated rows, the length of each row being 30 feet. Manuring, cultural operations, observation on the disease intensity and the calculation of average numerical score for each variety or culture were carried out according to the methods described by Padmanabhan and Ganguly (1959). Observations on the number and type of leaf-spots were taken once in the seedbeds on 30-day-old seedlings and then on the transplanted crops, 30-35 days after transplanting. For neck infections, the infected tillers were counted 25-30 days after flowering.

Forty-three varieties and two hundred and thirty cultures of paddy of various maturity groups collected from different regions of Chhota Nagpur were put to tests under field conditions of natural infection. Once a variety was observed as susceptible at any stage, it was finally classified as "susceptible."

RESULTS AND DISCUSSION : On critical examination of three years infection data it was seen that, in general, the conditions were less favourable for infection at the seedling stage, as only four out of forty-three varieties were susceptible at this stage and out of these, four varieties, only two have shown susceptible reaction in more than one season. The conditions were generally favourable for infection at the tillering and neck-infection stages.

The following among the cultures tested showed moderately resistant reaction :—

R. M. 5, R. M. 6, H. L. 12, H. L. 13, H. M. 1, H. M. 9, H. E. 10, S. L. 4, P. L. 15, and P. L. 16. Only sixteen cultures were moderately susceptible and others were all susceptible.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The results of the varietal trials indicate that none of the vaireties could be rated as resistant at all stages to blast. The best performance was given by *Baigan bichi*. With moderately resistant reaction at the seedling and tillering stages and resistant reaction at the neck infection stage. There were, however, some varieties which were resistant at one stage but susceptible at another, for instance, the varieties showing resistant or moderately resistant reaction at the seedling-tillering stages, but susceptible or moderately susceptible reaction at the neck-infection stage were, PTB 10, BK 115, CH 1007, B 76-1 and Kolaba. The varieties showing susceptible or moderately susceptible reaction at seedling-tillering stage but resistant or moderately resistant at the neck-infection stage were, BK 36, 35/2352 and 37/2342.

The varieties of the medium *aman* group were moderately susceptible or moderately resistant; they did not show susceptible reaction at any stage. Most of the *aus* paddy varieties were susceptible or moderately susceptible but only one, viz. PTB 10, was moderately resistant at the seedling stage and resistant at the tillering stage but susceptible at the neck-infection stage. This important *aus* variety was rated as moderately resistant at Cuttack (Padmanabhan and Ganguly, 1959), and moderately susceptible at Kanpur, (Mathur and Mishra, 1961).

ACKNOWLEDGEMENT : The authors acknowledge with gratefulness the active co-operation of Messrs. J. N. Sinha and R. S. Singh. They are grateful to Sri S. C. Mandal, Principal and Regional Director, for providing the necessary facilities and to Dr. S. Ramanujam, Director of Agricultural Research, Bihar and Dr. S. Y. Padmanabhan, Mycologist, Central Rice Research Institute, Cuttack, for critically going through the manuscript.

Agricultural Research Institute
Kanke, Ranchi

REFERENCES

PADMANABHAN, S. Y. AND D. GANGULY, (1959) Breeding rice varieties resistant to blast disease caused by *Piricularia oryzae* Cav. 1. Selection of resistant varieties from genetic stock. *Proc. Indian Acad. Sci.* Sec. B. **50** : 289-304.

MATHUR, R. S. AND M. P. MISHRA, (1961) Resistance of paddy varieties to blast in Uttar Pradesh. *Curr. Sci.* **30** : 272-273.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological Notes

A new blight disease of brinjal caused by Myrothecium roridum Tode ex Fr.—RAM NATH, M. L. SETH AND S. P. RAYCHAUDHURI, Indian Agricultural Research Institute, New Delhi.—In September 1965, some seedlings of brinjal (I. C. 10844) were observed showing light coloured circular spots of various sizes on the leaves with numerous black coloured pin-head-sized fructifications on both the surfaces. Microscopic examination revealed the presence of septate, hyaline and highly branched mycelium which ramified through these spots both inter-as well as intracellularly. The conidiophores emerged out of the diseased surface in groups and branched 3-4 times, forming a hymenium-like layer. The phialids were arranged in whorls of 3-4 or some times more and measured 10.5—15 0 μ x 2.0—3.0 μ. The conidia were produced at the tips of the phialids and were held together in a gelatinous black-coloured mass. They were single-celled, cylindrical, biguttulate with blunt ends, and were hyaline to light green with dark coloured walls and measured 5.0—7.0 μ x 1.5—2.0 μ.

The fungus was isolated from the surface-sterilized diseased spots on Potato-Dextrose Agar. It grew as whitish to very light coloured mycelium. Fructifications developed in concentric rings which changed colour from ash to green and finally to jet black. Mature synnemata were discoid or irregular with a distinct white rim of mycelium. No setae were observed either in diseased spots on leaves or in the culture. Single-spore isolations were made and the culture was maintained on Potato-Dextrose Agar because of good sporulation on this medium.

Two-month old seedlings of brinjal (I. C. 10844) were raised in the glasshouse for testing the pathogenicity of the fungus. The plants were inoculated with spore suspension in sterilized water. Some seedlings were also inoculated by putting the mycelium and the spore mass from the culture on stem, petioles and both sides of the leaves with the help of a sterilized brush These seedlings were immediately sprayed with sterilized water. All the inoculated seedlings were kept in humid chambers for 36 hours.

Within 24 hours of inoculation, small circular oil-soaked spots appeared on the leaves, petioles and stem of the seedlings which had been inoculated by spraying with spore suspension. Spots on the leaves became enlarged and brown coloured with distinct zonations, which finally coalesced covering most of the leaf surface (Fig. 1.). Some of the seedlings showed severe veinal necrosis resulting in the curling of such leaves. The lesions on petioles and stem became elongated, necrotic and sunken. In 5-7 days, leaves dried and dropped. Within 2—3 weeks, most of the seedlings were killed.

Seedlings which were inoculated with mycelium and spore-mass of the fungus developed comparatively larger oil-soaked irregular areas on

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Fig. 1. Brinjal plant showing blight symptoms produced by *Myrothecium roridum*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

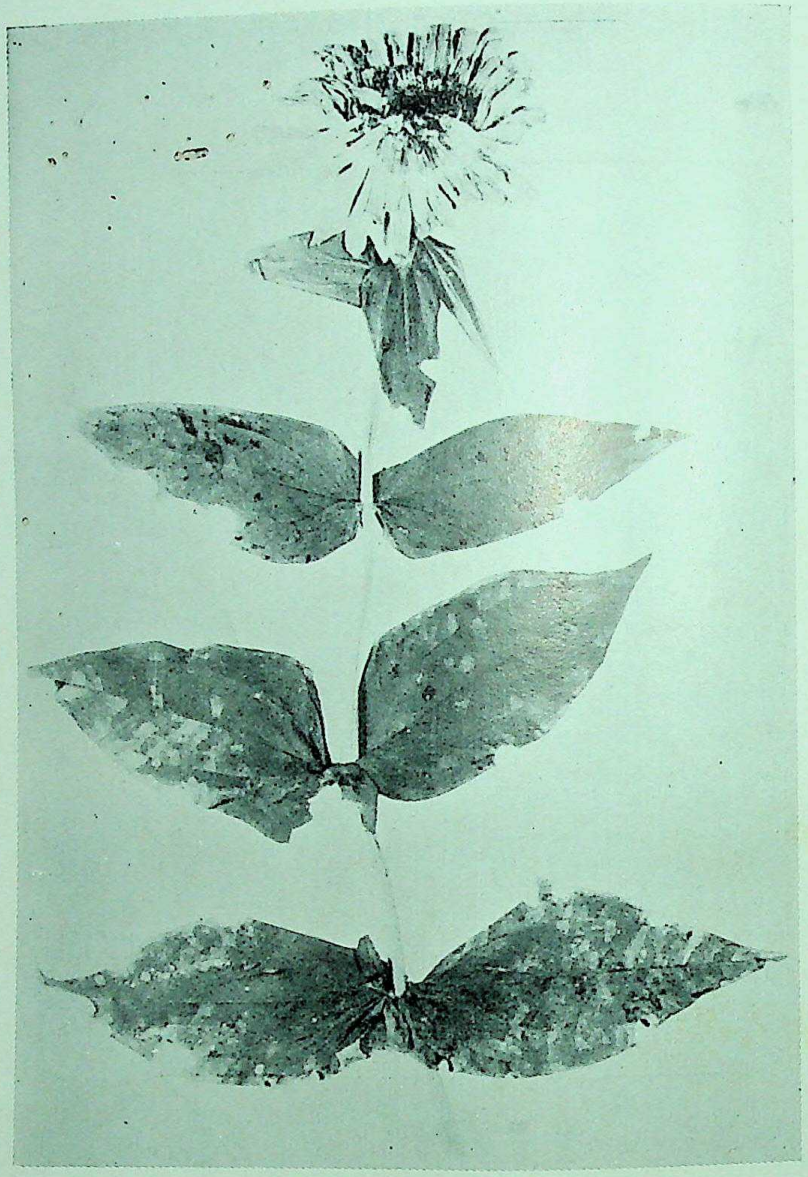


Fig. 1. *Zinnia elegans* showing dark-brown spots caused by infection with *Aphlenchoides ritzemabosi*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

the leaves without forming distinct spots and also developed severe necrosis of petioles and stem.

The fungus could easily be re-isolated from all the affected plant parts i. e. leaf spots, petiole and stem lesions.

The fungus has been identified as *Myrothecium roridum* Tode ex Fr. which has already been reported from India on several other hosts (Padmanabhan[1], Thirumalachar and Mishra[2]: Arya[3] and Munjal[4]), but has not so far been reported on brinjal, which is an important vegetable crop.

Acknowledgements : Thanks are due to U.S.D.A. authorities for providing the necessary funds as a P.L. 480 project for improvement of Vegetables, under which this work was carried out.

---

[1]Padmanabhan, S. Y. *Curr. Sci.* **18** : 56-57, (1948).

[2]Thirumalachar, M. J. and J. N. Mishra, *Pl. Prot. Bull.*, I (10) 145-146 ; 2 (1) 11-12, (1953).

[3]Arya, H. C. *Ind. Phytopath.* **9** (2) : 174-181, (1956).

[4]Munjal, R. L. *Ind. Phytopath.* **13** (2) : 150-155, (1960).

Aphelencholdes Ritzemabosi Parasitic on Zinnia elegans at Simla, India.—S. T. AHMAD, SHEODHAN SINGH AND D. P. MISRA, Indian Agricultural Research Institute, Plant Pathological Sub-station, Flowerdale, Simla—During the rainy season of 1964 several varieties of Zinnia. grown at the Plant Pathological Sub-station of Indian Agricultural Research Institute, Simla were observed to be affected with leafspots. Subsequent surveys in and around Simla showed that the disease was quite common in that locality. The initial symptoms appeared as small golden-yellow spots which were mostly rectangular in shape. The spots later turned brown to dark-brown and coalesced to form larger spots. Usually the cells in the affected areas were found to be killed and the leaves developed brown areas delimitted by veins (Fig. 1). Occasionally the spots were so numerous that a part or the whole of the leaf turned brown giving a scorched appearance to the plants. Often the spots along the margins became brittle and after their shattering away the leaves looked tattered. In general, the infected plants were stunted and died early. Diseased portions of the leaf when excised and left for a few minutes in water in a watchglass released nematodes. The morphological studies showed that the nematodes belonged to a species of *Aphelenchoides*.

Zinnia seedlings were grown in pots in a glasshouse up to 2-4 leaf stage. Leaves of healthy plants were kept immersed in a freshly prepared suspension of living nematodes for about 10 hours in bright day light. After about 6 days, characterstic symptoms developed on the inoculated leaves when the plants were kept at a temperature range of 12-20° C. Diseased portions of the leaves on examination showed the presence of nematodes similar to those used in the inoculations. The population of the nematodes was found to be very vigorous in the diseased material.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

This information, therefore, gave a clue that Zinnia was a congenial host for this nematode.

In order to determine the mode of spread of the disease under natural conditions, a separate experiment was laid out. Zinnia plants were grown in two separate pots. Plants in one pot were inoculated with a freshly prepared suspension of the nematodes, while the plants of the other pot served as contols. After the disease developed in the inoculated plants, the other pot in which healthy plants were growing, was kept in close proximity of the former, thus providing a chance of frequent contact between diseased and healthy leaves. These plants were sprayed with water once or twice daily until the disease symptoms started developing on the healthy plants. Diseased symptoms were obsered on healthy plants within a week of the start of the experiment. It, therefore, appears that humidity, even for a short time, enables the parasite to cause infection through contact. Zinnia being a monsoon annual, the climatic conditions and the planting pattern appear to be conducive to the spread of the disease in nature.

Morphological Characters of test species of the Aphelenchoides

♀ : 780-1050 μ ; a=45-47 ; b=10-14 ; c=19-21 ; V=67-74 per cent.

♂ : 750-950 μ ; a=40-50 ; b=8-12 ; c=17-20.

Body smooth, slender, tapering near extremities, lip region expanded, lateral field marked by 4 lines. Stylet 10-14 μ long with small and distinct knobs. Oocyst arranged in 2 or more lines. Tail uniformly tapering to a peglike point bearing bristles. Male tail forms a semicircle when relaxed by gentle heat.

On the basis of the above morphological characters, the test species of *Aphelenchoides* compares very well with *A. ritzemabosi*. Zinnia has been reported to be infected with *A. fragariae* (Ritzema-Bos) Christie (Halsted [1]) ; *A. ritzemabosi* (Schwartz) Steiner (Fukushi and Saito)[2] and *A. besseyi* Christie (Sher)[3] from various countries of the world. However, there is no record so far of any nematode parasitising Zinnia in this country, and apparently this happens to be the first record.

ACKNOWLEDGEMENTS : The authors are grateful to Dr. B. L. Chona, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his encouragement during the course of these studies. Thanks are also due to Dr. Gopal Swarup, Plant Pathologist (Nematology) for his helpful suggestions. The cooperation of Drs. B. B. Nagaich and K. K. Nirula, Central Potato Research Institute, Simla, is also acknowledged.

[1]Fukushi, T. and H. Saito *Trans. Tottori Soc. Agric. Sci.* 2 : 45-56, (1930).

[2]Halsted, B. D. *Gdn. & For.* 3 : 499, (1890).

[3]Sher, S. A. *Plant Dis. Reptr.* 38 : 687-689, (1954).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Association of the fungus Fusarium moniliforme Sheld. with the malformation in mango (Mangifera indica L.)— A. S. SUMMANWAR. S. P. RAYCHAUDHURI AND S. C. PHATAK*, Division of mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.— The malformation of mango is a serious disease of national importance. The symptoms of the disease are characterised in adult trees by the transformation of the inflorescence into a compact mass of sterile flowers. The vegetative symptoms are prominent by the production of numerous vegetative shoots at the apical meristem or at the axillary leaf (bunchy top). Most of the varieties are affected by this disease. This disease has been reported from almost all the mango-growing areas in India.

The constant association of an eriophyid mite and also various species of predatory mites in the malformed inflorescence have been reported by several workers (Narasimhan, 1954, 1959; Singh, 1955; Narayanan and Ghai, 1961). The role of eriophyid mites in producing the bunchy top disease in mango seedlings was further emphasized by Puttarudriah and Channa Basavana (1961). Nariani and Seth (1962) also reported the association of eriophyid mites in causing the malformation disease in mango.

The present studies were carried out in order to find out whether the disease had any association with any pathogenic fungi or bacteria.

During 1965, isolations were carried out on potato-dextrose agar (PDA) slants from the flower buds and the floral axis of malformed as well as healthy inflorescences of Desi, Chausa and Dashehri mangoes obtained from different places of the Indian Agricultural Research Institute estate. Precautions were taken to remove the scales/bark, after disinfection with 0.1 per cent mercuric chloride, before culturing the tissue. A fungus, identified as *Fusarium moniliforme* Sheld., was isolated from all the malformed tissues collected. No organism, however, could be isolated from the healthy portions of the same mango trees. The fungus is being maintained. Preliminary pathogenicity tests were undertaken.

Inoculations with *F. moniliforme* were carried out on healthy, 8to10 month-old, seedlings maintained in the glasshouse. These seedlings, except controls, were inoculated with the fungus isolated from the malformed tissues by the following inoculation techniques. The growing points of all the seedlings, including controls, were cut. (i) In the first set of experiment, a longitudinal incision of 3/4 to 1″ was made in the seedlings at the apical end. The culture was inserted in this incision and absorbent cotton, soaked in sterilized water, was placed over the cut end. Only wet absorbent cotton was placed on the cut ends of the control seedlings. The cut ends were then covered with polyethylene film to maintain moisture. The covers were removed after 3 weeks from the date of inoculations. (ii) In the second set, a sub-apical portion (2″ from the top) was inoculated after longitudinal injury. Wet absorbent cotton and polyethylene film were placed and removed, as described above.

---

* Division of Horticulture, Indian Agricultural Research Institute, New Delhi.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The malformed shoot appeared (Fig. A), 1½ months after inoculation, in the leaf axil on 5 out of 7 and 2 out of 3 mango seedlings in the first

and the second experiments, respectively, whereas, the controls produced healthy shoots (Fig. B). Reisolations from the induced malformed shoots yielded the same fungus. No fungal growth was observed when tissues from the control plants were cultured. Thus, the pathogenicity of the fungus (*F. moniliforme* Sheld.) was established under glasshouse conditions.

During the course of these experiments, precautions were taken to keep the young mango seedlings free from mites by spraying them with miticide (Aramite 15W, 0.1 per cent) once a week. Also frequent observations, with binoculars (dissecting microscope), were made to be sure that no mite was present.

This is the first record where the association of the fungus, *F. moniliforme* Sheld., with malformed mango shoots and inflorescences has been established. Further work to determine (i) if *F. moniliforme* is directly responsible for the disease, (ii) whether spores of this fungus are carried by mites, or (iii) whether *F. moniliforme* acts as a vector of a virus is in progress.

ACKNOWLEDGEMENTS : The authors are thankful to the Head of the Division of Horticulture, Indian Agricultural Research Institute, New Delhi, for supplying healthy mango seedlings for experimental work. Thanks are also due to Dr. S. B. Mathur, Pool Officer, Division of Mycology and plant Pathology, Indian Agricultural Research Institute., New Delhi, for his co-operation and to Dr. J. A. Von Arx, Director, Centralbureau Voor Schimmelcultures, Baarn, (Netherlands), for identifying the fungus.

[1]Narasimhan, M. J. *Curr. Sci.* 23: 297, (1954).

[2]————————. *Curr. Sci.* 28: 254, (1959).

[3]Narayanan, E. S. and S. Ghai. *Proc. 48th Indian Sci. Cong., Roorkee Part III* (Abstr.), (1961).

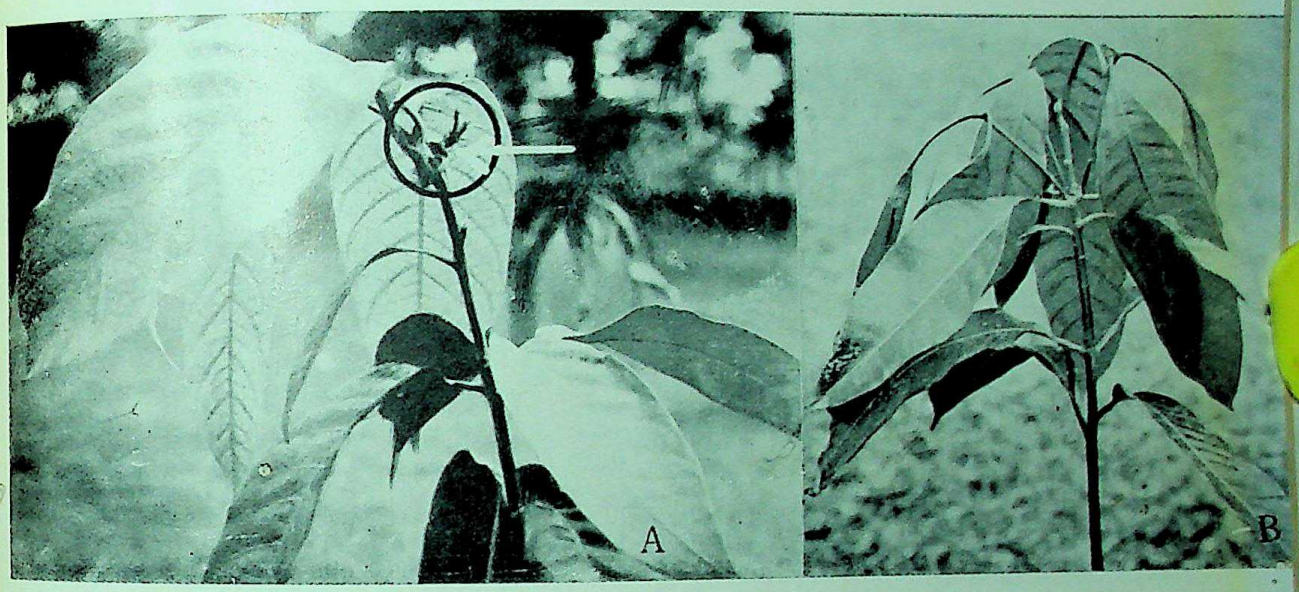
[4]Nariani, T. K. and M. L. Seth. *Indian Phytopath.* 15 : 231, (1962).

[5]Puttarudriah, M. and G. P. Channa Basavana. *Curr. Sci.* 30:114, (1961).

[6]Singh, S. N. *Curr. Sci.* 24: 168, (1955).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri



Young mango seedlings :

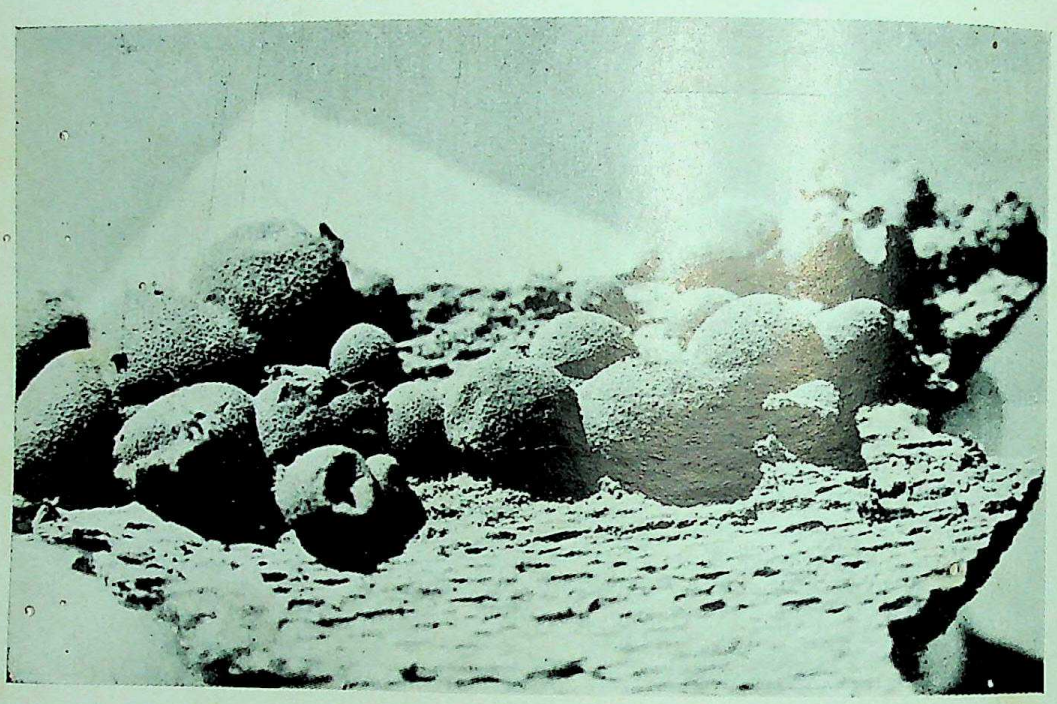
Fig. A Induced malformed shoot (arrow), 1½ months after inoclution with *F. moniliforme*

Fig. B Normal shoots arising on the control seedlings

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

AGNIHOTRUDU : LYCOGALA MYSORENSIS



Sporangia of *Lycogala mysorensis*

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Lycogala mysorensis (Agnihothrudu) Comb. nov. (syn. Licea mysorensis Agnihothrudu.)—V. AGNIHOTHRUDU, (Rallis India Limited, Fertiliser and Pesticides Division, 6-A, Cunningham Road, Bangalore—1. *Licea mysorensis* was described by Agnihothrudu (1965) from Ossoor coffee estate in Mysore. In the type collection, the fructifications were typically sporangiate, sessile with very scanty pseudocapillitium and hence, the collection was assigned to the family Liceaceae under the order Liceales.

Futher collections of this myxomycete from at least two more coffee estates in the Chickmagalur district were examined, In these, the fructifications were typical aethalia with more abundant pseudocapillitium than in the type collection. These characters which are closer to Reticulariaceae than to Lycogalaceae tend to reassign the fungus to the genus *Lycogala* and not *Licea*. Although the fructifications in all these collections are reminiscent of puff-balls, I could not, however, notice the interlacing tubular structures of the peridium which pierce the homogeneous inner layer and merge with the tubes of the capillitium that characterise the genus *Lycogala*. The spores in all my collections are dingy, pale olivaceous-green and never grey or pinkish. The pseudocapillitium does suggest *Lycogala* but it is different from that of any of the known species, so also do the spores; hence the new combination *Lycogala mysorensis* (Agnihothrudu) Agnihothrudu is proposed.

I wish to thank Prof. Dr. G. W. Martin, Emeritus Professer of Botany, Iowa State University, for his valuable suggestions and Mr. P. de Jong, General Manager, Fertilisers and Pesticides Division, Rallis India Limited, for permission to publish this note.

---

[1]Agnihothrudu, V., *Indian Phytopath.* 18 : 92-94, 1965).

Studies on the Host-Range of Uromyces Fabae (Pers.) De Bary—R. G. KAPOORIA AND S. SINHA, Botany Department, Agra College, Agra—Several species of *Vicia*, *Lathyrus* and *Pisum* have been found susceptible to *Uromyces fabae* (Pers.) de Bary in India (Uppal,[1] and Prasada and Verma,[2]) and abroad. Kapooria and Sinha[1] noticed natural infection on *Vicia sativa* L. and *V. hirsuta* Gray, the most common weeds in the fields of lentil (*Lens culinaris* Medic.) near Agra. They also determined their susceptibility to *Uromyces fabae* under glasshouse conditions. In the present paper, rust reactions on eight species of *Vicia* and *Lathyrus aphaca* L. have been presented.

Five to six seedlings of *Vicia* species and *Lathyrus aphaca* were inoculated with aeciospores of *Uromyces fabae*, following the usual methods of inoculation. Fifteen replications were maintained in each case. The disease symptoms appeared within ten days of inoculation at 16.7 to 36.7°C. The observations were recorded on the twefth day of inoculation.

The results reveal that *Vicia faba* L., *V. biennis* L., *V. hirsuta*, L. and *V. narborensis* L. are highly susceptible to *Uromyces fabae*, producing aecia on both the surfaces of the leaflets. *Vicia tetrasperma* Moench, *V. ervilia* Willd. and *V. gracilis* Loisal are also susceptible, though aecia developed only on the lower surface of the leaflets. *Vicia sativa* and *Lathyrus aphaca* were found to be resistant.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In the light of the studies reported herein, *Vicia faba*, *V. biennis*, *V. hirsuta* , *V. tetrasperma*, *V. narborensis*, *V. gracils* and *V. ervilia* can be expected to serve as collateral hosts for *Uromyces fabae*. Out of these hosts, *Vicia biennis*, *V. gracilis*, and *V. ervilia* are exotics and their indiscriminate introduction into India should be avoided.

[1]Kapooria, R. G. and S. Sinha. *Proc. 50th Indian Sci. Cong.*, Part III. (Abstr.) : 352 (1963).

[2]Prasada, R. and U. N. Verma. *Indian Phytopath.* I : 142-146. (1948)

[3]Uppal, B. N. *Int. Bull. Prot.* 7 : M. 103, and M 746. (1933)

Additions to the Fungi of Himachal Pradesh—H. S. SOHI AND G. K. GUPTA, Section of Plant Pathology, Department of Agriculture, Himachal Pradesh, Solan.—The paper deals with three new records of fungi for Himachal Pradesh, out of which *Microthyrium hederae* Feltg. is the first record for India.

Discosia artocreas (Tode ex Fr.) Fr., in Summ. veg. Sci. p. 423 ; Sacc. in Syll. Fung. 3 : 653, 1884., Chona et al., Indian Phytopath., 10 : 154, 1957 ; and Hasija, S. K. Mycopath. et Mycologia appl., 18 : 84, 1962.

On living leaves of *Ficus rep-ens* Rotts. (Urticaceae), 1500 m. m. s. l., Solan, Himachal Pradesh, March, 1965, G. K. Gupta, I. M. I. Herb. No. 114375.

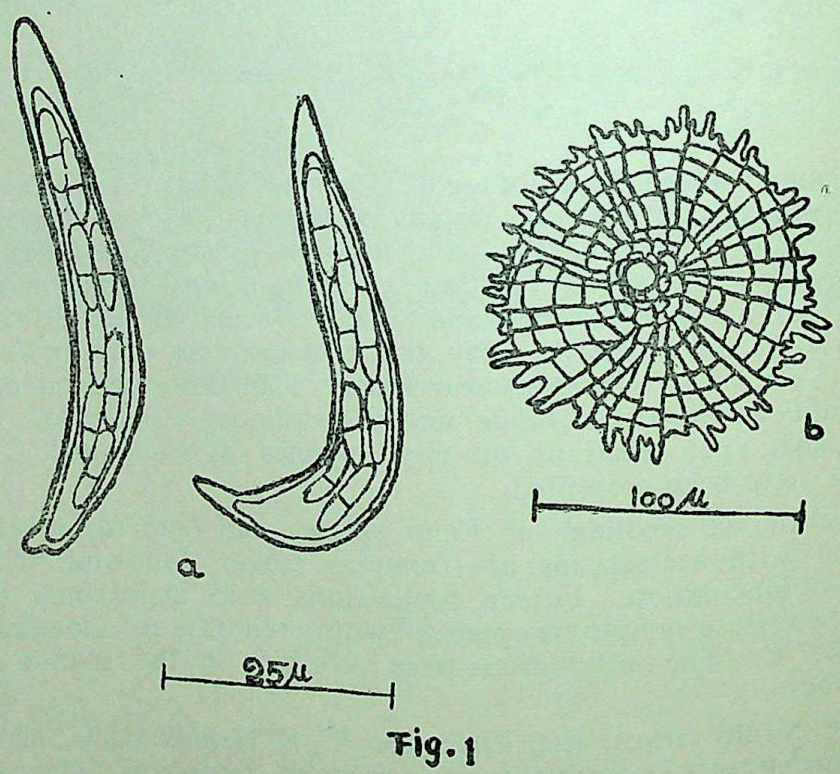


Fig. 1. *Microthyrium hederae* Feltg. a. Asci and Ascospores. b. Perithecium.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

The fungus forms ashy coloured leaf spots with distinct dark reddish brown margins, which are generally delimited by midribs of the leaves. The spots become papery and brittle and show the presence of black pycnidia. Ultimately, the middle portions of the spots fall off. Other characters of the fungus agree with the above species.

In India, this fungus has so far been recorded on *Pyrus communis* and *Terminalia* sp. The present report appears to be a new addition to the host index of this fungus.

Microthyrium hederae Feltg., Vorstud. pilz : Luxemb. Nachtv. 3 : 310,1903 ; Sacc., Syll. Fung., 17 : 863, 1905.

On living leaves of *Hedra helix* L. (Araliaceae), 1500 m. m. s. l., Solan, Himachal Pradesh, February, 1965, G. K. Gupta, I. M. I. Herb. No. 114376.

Spots circular to irregular, initially delimited by veins and veinlets with ashy centres on the upper surface of the leaves and greyish on the lower, young spots surrounded by thin purple-brown margins, spots coalesce with each other covering large areas of the leaf, centres of the spots later on become papery, brittle and fall off leaving wide holes.

Perithecia superficial, dimidiate with radiate scutellum, round, ostiolate, glabrous, 136.4-198.4 × 111.6-176.6μ, free mycelium lacking, hymenium single, asci hyaline, basal, clavate to obclavate, 8-spored, 36-51 × 6-9μ ; ascospores hyaline, oblong to elliptical, uniseptate distichus, 9-12 × 2.3-3 μ ; paraphyses absent.

Cercospora plumeriae Chupp., A monograph of the fungus Genus *Cercospora*, p. 49, 1953 ; Chowdhury, S. Lloydia, 21 : 152, 1958.

On living leaves of *Plumaria acutifolia* Poiret (Apocyanaceae), 540 m. m. s. l., Dhaula Kuan, Himachal Pradesh, January, 1965, G. K. Gupta, I. M. I. Herb. No. 114377 and 114383.

ACKNOWLEDGEMENTS : The writers are grateful to Dr. G. C. Ainsworth, Director, Mr. Pirozynski and Mr. Deighton of the Commonwealth Mycological Institute, Kew, for their help in the identification of these fungi and also to Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh, for his keen interest and encouragement during the course of this work.

Collar and root rot of 'ajowan'—A. S. PRABHU, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.—Ajowan (*Trachyspermum ammi* (L.) Sprague, Syn. *Carum copticum* Benth is an essential-oil-yielding spice plant cultivated chiefly in Madhya Pradesh, Andhra Pradesh, Gujarat, Maharashtra and Uttar Pradesh.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A disease causing collar and root rot was observed during March and April of 1964 and 1965 in the experimental fields of the Botany Division, Indian Agricultural Research Institute, New Delhi. The disease occurred in patches at the time of flowering when the mortality of the plants was about 15 per cent. Infection first appeared at the collar region and later extended to the entire root causing soft decay of the outer tissues on which a white fluffy mycelial growth of the fungus and sclerotia of the size of mustard seed developed. When the humidity was high the fungus formed a patch of white mycelial growth and sclerotia on the soil around the base of the plant.

Isolations made by planting surface-sterilized sclerotia and affected root tissue on potato-dextrose agar yielded a pure culture of the fungus which was identified as *Sclerotium rolfsii* Sacc.

To test its pathogenicity, inoculum was prepared by growing the fungus on autoclaved wheat seeds at 30°C for 5 days after which the infested seeds were air-dried. Two infested wheat seeds were placed at the collar region of each plant in the pots and kept in a moist chamber for 48 hours and later removed to glasshouse benches. Four plants, about one month old and raised in 6″ pots, were inoculated at a time and the experiment was repeated thrice. Uninoculated controls were maintained. The temperature during the experiment ranged between 30—35°C. The first symptoms of wilting were observed within 3 to 4 days of inoculation. The fungus mycelium surrounded the collar and the root of the affected plants.

In order to find out the age at which 'ajowan' is most vulnerable to attack, plants at different stages of growth, viz. 15 days, 1 month, 2 months and 3 months old were inoculated simultaneouly. The results of these tests have shown that the plants of all ages are equally susceptible suggesting that the occurrence of this disease at the time of flowering in March—April might be due to the temperature and moisture conditions prevailing during this period.

The optimum temperature for growth in culture was determined to be 30°C and above 35°C the fungus failed to grow in culture. Sclerotia stored in the laboratory for 6 months from May-March required 6 to 7 days' soaking in water for germination.

Aversion to growth and sclerotial formation were observed between some isolates of the fungus when grown on potato-dextrose agar in petri plates. Based on this phenomenon of aversion, which was first described by Nakata[1], four strains of the fungus affecting 'ajowan' were distinguished. Out of these, the strains S64 and S65 were morphologically distinct in that they showed differences in the number and the size of sclerotia. The isolate S65 produced a heavier mycelial mat and a smaller number of sclerotia which were smaller in size than those of S64. These two strains retained their identity even after passing back through the host. The other two strains S65-1 and S65-2 which exhibited aversion to both S64 and S65 and between themselves were morphologically indistinguishable and closely resembled S65.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In the host-range studies, the seedlings of *Triticum aestivum* L., *Hordeum vulgare* L., *Zea mays* L., *Sorghum vulgare* Pers., *S. vulgare* var. *sudanense* (Piper) Hitchc. were resistant, whereas *Vigna sinensis* Endl., *Phaseolus vulgaris* L., *Vicia faba* L., *Cucumis melo* var. *utilissimus* Dutchie and Fuller, *Citrullus vulgaris* Schrad., *C. vulgaris* var. *fistulosus* Stocks., *Cyamopsis tetragonoloba* (L.) Taub., *Solanum melongena* L. and *Lycopersicum esculentum* Mill. were found highly susceptible to S64 and S65. There was no apparent difference in virulence between these strains.

Collar and root rot is a new record of disease on 'ajowan' and a new host record for *Sclerotium rolfsii* which has more than 180 hosts[2] throughout the tropics of the world.

The author is grateful to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for his encouragement and for critically going through the manuscript.

---

[1]Nakata K. *Bulteno Sci-Fakultato Terkulture Kjusu Imperia Universitato* **1**: 177-190 (1925).

[2]Weber G. F. *Phytopathology* **21** : 1129-1140 (1931).

Influence of cultural practices on the population of Meloidogyne javanica (Treub, 1995) Chitwood, 1949.—R. B. S. BIRAT. Assistant Plant Protection Officer, (H. Qrs.), Bihar, Patna.—Evidence is available in literature that root-knot nematodes, *Meloidogyne* spp. survive in the soil for a few weeks even in the absence of their host plants. As soon as the host plant is detected, the larvae congregate around the growing root-tips and penetrate inside. A gradual increase in the nematode population inside the roots during their growth period and an abrupt decrease following their cessation on attaining maturity have been reported by earlier workers.

With a view to evolving a suitable cultural control of the root-knot nematodes, a micro-field trial was conducted. A heavily root-knot-infested field was selected for the purpose. The field was divided into five equal blocks and each block was further sub-divided into four similar plots, each measuring 5 × 3 square feet. The field was laid out in March, just after the harvest of susceptible tomato varieties. The treatments were randomized in each replicate. Under treatment (a) the plots were left without any cultural operation to serve as control; in treatment (b) the plots were dug nine inches deep with a spade twice in March and May respectively; in treatment (c) the plots were fallowed and the top six-inch soil was stirred every month from March to June; treatment (d) the plots were sown with a non-susceptible *Samma* (*Panicum crusagalli*) crop in March and harvested in June while under treatment (e) the plots were sown with a highly susceptible crop *bhindi* (*Abelmoschus esculentus* (L) M. From March onward till June twenty earthern pots were potted every month with the soil from all treatment plots, one pot from each plot, and healthy tomato seedlings (three per

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

pot) were grown in them for 30 days. The nematode population in respect of each treatment was determined by counting the galls on the test-plants. Finally in the month of June all the plots were given uniform cultural operations and healthy tomato seedlings were grown for another 30 days. At the end, a similar assessment was made to comprehend the population levels of the nematodes in the soil which could penetrate the roots and succeeded in forming galls. Sections of posterior cuticular patterns of ten adult females from each treatment were cut under the stereoscopic microscope and studied with the help of the key prepared by Taylor et al.[4] They revealed a wide occurrence of *Meloidogyne javanica*.

The experimental data are summarised in Table 1.

TABLE 1 : Population of *Meloidogyne javanica*, as influenced by cultural practices

	Treatments				
	Average number of galls per plant				
Months	Without cutural operation	Hot weather cultivation	Fallowing	Growing non-susceptible crop Samma	Growing susceptible crop bhindi
	(a)	(b)	(c)	(d)	(e)
March	55.00	57.25	52.00	55.25	57.00
April	40.25	14.25	33.25	21.75	64.20
May	38.50	10.25	21.25	15.75	106.25
June	36.00	7.75	16.50	12.00	118.00
July	103.00	10.75	27.25	22.00	120.35

It is obvious from the data in Table 1 that there is a sharp decline in nematode population from March to June in absence of suitable host plants in the field (under treatments b, c and d). The presence of susceptible host plants (treatment e) encourages heavy build-up of the nemic population, irrespective of high atmospheric temperature. A bare fallow without any cultural operation (treatment a) permits the maintenance of a population level potentially dangerous to cause severe damage to plants to be grown subsequently in the field, though there is a decreasing trend from March to June. The weeds growing in the plots provided conditions for the development of the parasite during March to June. Afterwards it remains active on bhindi, brinjal, tomato, beans, cucurbits and a host of other cultivated plants from July to February. If the field is kept free

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

from vegetation during this critical period either by the hot-weather cultivation or by fallowing, the nematode population declines to a considerable extent. Even the cultivation of non-susceptible hosts diminishes the nemic activities far less than the growing of susceptible crops.

This information can be utilised for the development of suitable cultural control in the form of hot-weather cultivation, summer fallowing and the growing of non--susceptible hosts alone or in combinations. Similar observations have been recorded by Godfrey and Hagan[1], Jacob[2] and Nusbaum[3] in other countries for the control of different species of *Meloidogyne*. In India this is the first attempt in this direction and provides a rich field for further investigations.

The author wishes to express his deep sense of gratitude to Dr. B. S. Lall, Professor of Entomology, B. A. College, Sabour, for his valuable guidance.

---

[1]Goodfrey, G. H. and H. R. Hagan. Preli. Rept. Pineapple Quart. (Hawai) **1** : 10-18. (1931).

[2]Jacob, J. J. *Nematalogica* Suppl. II, 141-143 (1960).

[3]Nusbaum, C. J., *Phytopathology* 49 : 547-548 (1959).

[4]Taylor, A. L., V. A. Dropkin and G. C. Martin, *Phytopathology* **45** : 26-34, (1955).

Powdery Mildews from Himachal Pradesh-I.—H. S. SOHI, S. L SHARMA AND K. B. SACHDEVA.—Section of Plant Pathology, Department of Agriculture, Himachal Pradesh, Solan.—During the course of periodic surveys of Government farms and orchards in the Pradesh, a number of powdery mildews were collected and examined, of which the following two powdery mildews recorded on four different hosts are new records for India. The specimens have been deposited in the Sectional Herbarium of Plant Pathology Section, Department of Agriculture, Himachal Pradesh, Solan and their respective numbers are indicated in the text.

1. **Pleochaeta shiraiana** (Henn.) Kimbr. & korf.

On living leaves of *Celtis australis* (Salicaceae), H. C. No. 6001, Solan, Himachal Pradesh, November-December, 1962 (H. S. Sohi).

This species differs from the one described by Lall, G. and Gupta J. N. (1965) in the measurements of conidia, cleistothecia, asci and ascospores, which in the present fungus were 37.2—66.4 × 10.4—20.9 μ, 230.6—327.2 μ. 76.8—111.8 × 20.9—31.4 μ and 22.1—39.6 × 13.9—20.9 μ, respectively. It also differs in having generally 3 spored asci and sometimes 4 spored asci whereas G. Lall and Gupta have described always having 3 spored asci

2. **Sphaeootheca macularis** (Wall) ex Fr.) W. B. Cooke var *fuliginea* (Schlecht ex Fr.) W. B. Cooke

On living leaves of *Phaseolus mungo* L. (Leguminosae), H. C. No. 6002, Solan, Himachal Pradesh, September-October, 1963 (H. S. Sohi).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Amphigenous, forms white irregular patches covering almost whole of the leaf surface, later on becoming dirty white; mycelium hyaline, septate ; perithecia gregarious, brown to dark brown in colour, globose to sub-globose, 65.2 – 90.8 × 55.9 – 83.8μ in diameter, cells irregular, 18.0–41.4 × 14.4–27.0 μ ; appendges variable in number and length, hypha-like, flexuous, sparse, brown, septate, mostly simple sometimes 1-2 times branched or irregularly bent ; ascus hyaline, single, 7-8 spored, subglobose to broadly ovate, without a stalk, 62.9–88.5 × 58.2–69.9 μ ; ascospores hyaline, one celled, broadly oblong to ellipsoidal, 18.6–23.3 × 16.3–20.9 μ.

Another record of this powdery mildew was made on leaves, stems and inflorescence of *Erigeron linifolius* Willd. (Compositae), H. C. No. 6006, Solan, Himachal Pradesh (H. S. Sohi), the description of which is as under :—

Mycelium white, forming white irregular patches on all parts of the plant ; conidia produced in chains, elongate-ellipsoidal or barrel shaped, hyaline, 25.6—51.2 × 13.9–19.8 μ ; perithecia scattered, globose to sub-globose, brown to dark brown in colour, 58.2–86.2 μ in diameter ; cells distinct, 11.6–20.9 μ wide ; appendages hypha-like, brown, septate, sometimes branched, variable in number and length, generally 1-2 times the diameter of the perithecium ; ascus single, hyaline, globose to ovate, sessile, 55.9–69.9 × 46.6–65.2 μ, 8-spored ; ascospores hyaline, ellipsoid, 16.0- 20.9 × 11.0–16.3 μ.

3. Erysiphe polygoni DC.

On living leaves, stems and inflorescences of *Chenopodium ambryosioides* L. (Chenopodiaceae), H. C. No. 6005, Solan, Himachal Pradesh, November, 1963 (B. R. Verma and S. L. Sharma).

Amphigenous, mycelium variable, persistent forming thin or rarely thick white irregular patches, often covering the leaves, stems and inflorescence, later on becoming dirty white, mycelium hyaline, septate, 3.4–5.8 μ wide ; conidia barrel shaped, hyaline, granular, 23.3–38.4 × 11.6–16.3 μ ; perithecia scattered, brown to dark brown in colour, globose to sub-globose, 81.5—137.4 μ in diameter, cells distinct, 9.3–16.3μ wide ; appendages hypha-like, brownish, septate, 1-2 timesthe diameter of the perithecium, irregularly bent, sometimes branched ; asci 6–7, sub-globose to broadly ovate, short to long-stalked, hyaline, 51.2–88.5×30.2–46.6 μ; ascospores 3–5, hyaline, oval to oblong, one-celled, 16.3–28.5 × 10.4–16.3 μ.

This mildew was also recorded on living leaves of *Clematis grata* Wall. (Renunculaceae), H. C. No. 6007, Solan, Himachal Pradesh, November-December, 1964 (H. S. Sohi).

Amphigenous, forming white irregular patches on both surfaces of the leaves ; mycelium hyaline, septate, 2.7–3.6 μ wide ; conidia barrel-shaped, hyaline, granular, 23.4–46.8×9.0–18.0 μ ; perithecia scattered,

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

brown to dark brown in colour, globose to sub-globose, 85.5 – 131.6 μ in diameter ; cells not distinct, irregular, 14.4 – 28.8 × 10.8 – 21.6 μ ; appendages flexuous, intermingled with the mycelium, sometimes not distinguishable from it, 4.5 – 7.2 μ wide and 5 to 6 times the diameter of the perithecium ; asci 6 – 7, sub-globose to broadly ovate, sessile to short-stalked, hyaline, 46.8 – 70.2 × 30.6 – 47.7 μ ; ascospores 3 – 5, hyaline, oval to oblong, one celled, 16.2 – 23.4 × 9.9 – 14.4 μ.

ACKNOWLEDGEMENTS : The authors are highly grateful to Dr. L. S. Negi, Director of Agriculture, Himachal Pradesh, Simla, for his keen interest and encouragement during the course of these studies.

---

[1] *Pleochaeta shiraiana* (Henn.) Kimbr. and Korf. in Mycologia, **55** : 624, 1963 ; Lall, G. and J. N. Gupta. *Indian Phytopath.* **18** : 213, (1965).

[2] *Sphaerotheca macularis* (Wall. ex. Fr.) W. B. Cooke var. *fuliginea* (Schlecht ex Fr.) W. B. Cooke in *Mycologia*, **44** : 570-74, (1952).

[3] *Erysiphe polygoni* DC. in Salmon Monograph Erysiphe 174, 1900 ; *Bull. Torr. Bot. Club.* **27** : 443, 1900 ; and **29** : 181, (1902).

Influence of soil temperature on infection of sugarcane by the smut fungus Ustilago scitaminea Syd.— A. K. LAMBAT, V. V. CHENULU AND B. L. CHONA, Division of Mycology and Plant Pathology, Indian Agricultural Reserach Institute, New Delhi.— In any programme of screening varieties for resistance to diseases, as many of the conditions as possible for maximum disease development should be provided. In the testing of sugarcane varieties for resistance to smut caused by *U. scitaminea*, the influence of factors such as viability of the inoculum, inoculation method, spore load or density, variation in soil moisture and the selection of proper seed material on infection have been investigated by various workers from time to time (Luthra *et al*[5]., Harbans Singh *et al*[3]., Mohan Rao and Prakasam[6]).

However, no attention has been paid to the role of soil temperature on the infection of sugarcane by the smut fungus. The influence of soil temperature on the infection of crop plants by smut fungi has been well established by Faries[2] in the case of oat smut, Stakman *et al.*[7] in rye smut and Adlakha and Munjal[1] in the case of grain smut of sorghum. In the last case, by providing optimal soil temperature for 3 weeks following inoculation the percentage infection has been raised from zero to 70 in some varieties.

An experiment was, therefore, conducted to find out the optimal soil temperature for infection in the early stages of infection of sugarcane by the smut fungus and the results are reported in this communication.

Two susceptible varieties of sugarcane viz. Co. 1212 and Co. 1279 were selected for the study. A standard spore suspension was prepared by mixing 0.5 gm. of spores of the fungus in two litres of water. The setts selected from the top 1/3 part of canes were steeped in the spore suspension for 30 minutes after which they were planted in 4″ pots filled with sterile soil and watered to the maximum soil-saturation capacity. The

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

pots were kept inside a Gallenkamp temperature-cum-humidity-controlled chamber, adjusted to different temperatures ranging from 20 to 35°C. Twenty one-budded setts of each variety were exposed after inoculation to different temperatures for one week. The actual soil temperature in the pots was accurately read by means of a thermometer. At the end of a week's exposure to different temperatures, the setts were removed, replanted in 14″ pots filled with sterile soil and kept outside for further observations. A period of one week's exposure to different soil temperatures was chosen because the early phases of infection, i. e. germination of the spores, penetration of the host and establishment within the host is expected to be completed within this period and once inside the host the pathogen is not much influenced by the external soil temperature for its development. Disease incidence counts were taken at weekly intervals up to 30 weeks after planting. Setts that failed to produce smut whips at the end of 30 weeks' period were treated as not infected. The results are presented in Table 1.

It is evident from the results, that a soil temperature of 25°C. is optimal for infection of sugarcane by the smut fungus. Incidentally, a temperature of 25°C. is optimal for germination of the fungal spores (Lambat)[4] indicating that the effect is on the pathogen. These results point out that the incubating of the setts at 25°C. following inoculation for at least a week before planting them in the field will appreciably increase infection.

TABLE 1: Influence of soil temperature on the infection of sugarcane varieties Co. 1212 and Co. 1279 by the smut fungus *Ustilago scitaminea* Syd.

Variety	Percent smut infection at			
	20°C.	25°C.	30°C.	35°C.
Co. 1212	*50	75	60	20
Co. 1279	45	65	55	15

*Based on 20 setts for each variety

The authors are grateful to Dr. S. P. Raychadhuri, Head of the Division of Mycology and Plant Pathology for providing necessary facilities and for encouragement during the course of the investigation. The senior author is also thankful to the Indian Agricultural Research Institute for the award of a fellowship from 1962 to 1963 during which period this work was completed.

[1]Adlakha, K. L. and R. L. Munjal, *Ind. Jour.* of *Agric. Sci.*, **33** : 8-10. (1963).

[2]Faries. J. A. *Amer. Jour. Bot.* **11** : 189-214. (1924).

[3]Harbans Singh, Sardul Singh and Nand Singh. The 3rd All-India Conf. of Sugarcane Research and Development Workers in India. pp. 362-65. (1959).

[4]Lambat, A. K. Ph. D. Thesis submitted to P. G. School of I. A. R. I. (1965).

[5]Luthra, J. C., A. Sattar, and S. S. Sandhu, *Proc. Indian Acad. Sci., Sect. B*, **XII**, 118-120. (1940).

[6]Mohan Rao, N. V. and P. Prakasam, *Proc. Int. Soc. Sug. Tech.* 9th Congress 1048-57. (1956).

[7]Stakman, E. C., M. B. Moore, and R. C. Cassell, *Phytopathology* **24** : 18 (Abs.) (1934).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

A Preliminary note on the Plant Parasitic Nematodes of the Mysore State.— B. C. N. SWAMY AND H. C. GOVINDU, Division of Plant Pathology, Agricultural Research Institute, Hebbal, Banglore-24, 20th March 1965— Except for stray reports on the occurrence of plant parasitic nematodes from the Mysore State, there has been no attempt to make a systematic study of this important group of plant pathogens which are known to cause considerable damage to many of our economic crops especially food, fruit, vegetable and ornamental plants. A critical survey was undertaken in this Division during the years 1963-1965, with a view to collecting and studying the extent of damage, life-history and to evaluating control measures. In the present paper 12 genera, 16 species and 1 variety of nematodes are reported on 52 hosts.

Diseased plants and soil around the roots were collected from different parts of the State. They were examined in the laboratory for the presence of parasitic nematodes. The nematodes were isolated by employing modified Baermann funnel technique developed by Stoller[2]. The identification of *Meloidogyne* spp was based on the key given by Taylor *et al* [3]. The rest of the nematodes were identified by consulting Goodey's "Soil and Fresh water nematodes".

Among the several species of namatodes studied, root-knot causes the maximum damage on crop plants, particularly vegetables. As can be seen from the list, three species and one variety of *Meloidogyne* were collected from 38 out of 52 hosts studied. This clearly indicates the wide host-range of the root-knot nematodes.

The genera *Aphelenchoides*, *Helicotylenchus*, *Hirschmaniella*, *Hoplolaimus*, *Meloidogyne*, *Nothanguina*, *Pratylenchus* and *Trichodorus* were found in abundance; on the other hand, the genera *Aphelenchus*, *Criconema*, *Heterodera* and *Xiphinema* were found in lesser numbers.

The following is the list of plants on which the nematodes have been reported :—

1. (a) *Aphelenchoides* sp. causing dark patches on the leaves of *Bellis perennis* Linn., *Chrysanthemum indicum* L. and *Viola* sp.

(b) *A. asterocaudatus* Das on the bud and leaves of *Allium sativum* L.

(c) *A. parietinus* (Bast.) Steiner in soil around the roots of *Eleusine coracana* L. Gaertn., *Gossypium hirsutum* L. and *G. herbaceum* L.

2. *Aphelenchus* sp. in soil around the roots of *Gossypium hirsutum*.

3. *Criconema* sp. in soil around the roots of *Phaseolus vulgaris* L.

4. *Helicotylenchus* sp. in soil around roots of *Areca catechu* L. *Capsicum annuum* L. *Eleusine coracana*, *Gossypium herbaceum*, *G. hirusutum* and *Phaseolus vulgaris* L.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

5. *Heterodera cacti* Filip and Stek. on the roots of *Echinopsis* sp.

6. *Hirschmaniella oryzae* (V. Breda de Haan) Luc and Goodey in the roots of *Oryza sativa* L.

7. *Hoplolaimus* sp. in soil around roots of *Gossypium arboreum* L., and *Musa paradisiaca* L.

8. (a) *Meloidogyne arenasia* (Neal) Chitwood on the roots of *Arachis hypogea* Willd., *Capsicum annuum*, *Citrullus vulgaris* Scharad., *Coleus blumei* Benth., and *Nicotiana tabacum* L.

(b) *M. incognita* (Kofoid & White) Chitwood on the roots of *Allium cepa* L. *Anethum graveolens* L. *Beta vulgaris* L. *Carthamus tinctorius* L. *Cucurbita maxima* Duch., *Daucus carota* L. *Echinopsis* sp., *Gossypium barbadense* L. *Morus alba* L. *Musa paradisiaca*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Raphanus sativus* L. *Solanum nigrum* L. and *S. tuberosum* L.

(c) *M. incognita* var. *acrita* Chitwood on the roots of *Capsicum annuum*, *Cosmos sulphureus* Cav., *Lactuca sativa* L. *Lycopersicum esculentum* Mill. and *Pogostemon patchouli* Pellet.

(d) *M. javanica* (Treub) Chitwood on the roots of *Abelmoschus esculentus* (L.) Moench., *Amarantus gangeticus* L. *Bellis perennis* L. *Basella rubra* L. *Brassica oleracea* var. *capitata* L. *Celosia argentia* L. *Conyza ambigua* DC., *Eclipta prostrata* (L.) *Gomphrena decumbens* Jacq., *Imptiens balsamina* L. *Lycopersicum esculentum*, Mill *Pisum sativum* L. *Saccharum officinarum* L. *Sesbania bispinosa* (Jacq.) W. F. Wight, *Solanum melongena* L. and *Vernonia* sp.

9. *Nothanguina cecidoplastes* (Goodey) Whitehead, causing purple coloured galls on the leaves of *Bothriochlea pertusa* (Willd.) A. Camus and *Dicanthium annulatum* Stapf.

10. *Pratylenchus* sp. in the roots of *Cocos nucifera* L. *Dolichos lablab* var. *typicum* Prain, *Eleusine coracana*, *Gossypium herbaceum* and *Saccharum officinarum*.

11. *Trichodorus* sp. around the roots of *Eleusine coracna* and *Saccharum officinarum*.

12. *Xiphinema* sp. in soil around the roots of *Citrus aurantifolia* (Christm) Swingle.

A detailed study of the nematodes, in general, and their biology and control aspects, in particular, are in progress.

---

[1]Goodey, J. B. "Soil and fresh water nematodes" 1963, Methuen & Co. Ltd. London. pp. 544.

[2]Steller, B. M. *Plant Dis. Rept.*, **41**, 531 (1957),

[3]Taylor, A. L., V. H. Dropkin, and G. C., Martin. *Phytopathology* 45, 26-34, (1955).

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Antifungal activity of streptomycin.—M. R. S. IYENGAR, Microbiological Research Laboratory, Alembic Chemicals, Baroda.—We have employed microgram quantities of streptomycin to provide some protection to penicillin fermentations[1], both in the seed and the production stages. At the seed stage, the antibiotic is generally added separately from the spore inoculum. However, in a few experimental runs when the antibiotic was added together with the spore inoculum, the growth of the strain of *Penicillium chrysogenum* Thom. was markedly inhibited. Whatever growth was observed consisted of small numbers of large pellets. In strains of the fungus which give filamentous growth at high spore loads such effects are generally observed when the spore load is very small[2] This observation indicated that large numbers of the spores were possibly killed because of contact with streptomycin.

The concentration of streptomycin in the spore suspensions mentioned ranged between 4.0 and 6.0 mg. (as sulphate) per ml. and the time of contact, between half to three-fourths of an hour. In further experiments, a sterile distilled-water suspension of washed *P. Chrysogenum* spores from one-week to ten-day-old culture was mixed with a solution of streptomycin sulphate, also in sterile distilled water, to give 1×10^6 spores and 5.0 mg. of streptomycin sulphate per ml. After a contact period of one hour, the viability of the spores was checked by planting on honey-peptone agar, using five replicates. Suitable controls without sterptomycin were also used. Tests were run in the same manner using cells from a one-day-old culture of a strain of baker's yeast, *Saccharomyces cerevisiae* Hansen and a strain of *Candida intermedia* (Cif. et Ashf.) Langeron et Guerra on Sabouraud's agar. The results from several tests (Table) confirmed that streptomycin sulphate had a marked fungicidal effect under the conditions employed.

TABLE : Fungicidal activity of streptomycin sulphate*

P. chrysogenum	66.2	90.0	64.0	90.0
S. cerevisiae	..	81.0	91.9	72.4
C. intermedia	...	...	90.0	90.0

*Values expressed as percentages of controls without streptomycin.

When tested at lower concentrations against *P. Chrysogenum*, streptomycin sulphate gave the following percentages of sporicidal activity in three separate experiments involving three different batches of the antibiotic : 49.8, 34.0, and 32.0 at 500 μg/ml, and 0.0, 32.4 and 0.0 at 50 μg/ml.

The streptomycin sulphate used in these experiments was of U.S.P./B.P. grade. There were indications that different batches of the antibiotic with equal base potency had significant differences in their fungicidal effect, as determined by the *P. Chrysogenum test*.

Streptomycin is generally considered to be inactive against fungi *in vitro*[3] and, to our knowledge, direct fungicidal activity has not been ascribed to streptomycin preparations. The results presented show that streptomycin is fungicidal *in vitro* depending on the concentration used.

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

In several fungal diseases of plants the use of streptomycin has yielded beneficial results. The observed effects are generally considered to be indirect and, in one case, evidence has been presented to show that these effects are possibly related to the antibiotic-induced increase of polyphenolase activity in the plant tissues[4]. The fungicidal effects presented here indicate a need to critically re-examine the possibility of direct antifungal action of streptomycin preparations on the pathogens concerned under different conditions, and further assess the relative contributory roles of direct and indirect effects.

---

[1]Indian patent 63760, (1958).

[2]Thirumalachar, M. J. and K. S. Gopalkrishnan. Antib otics, a symposium 80-82, Council of Scintific and Industrial Research, New Delhi. (1956)

[3]Waksman, S. A., and H. A. Le Chevalier. *The Actinomycetes* Vol. III: 374-375. The Williams and Wilkins Co., Baltimore (1962).

[4]Voros, J., Z. Kira'ly, and G. L. Farkas. *Science* **126** : 1178. (1957)

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

BOOK REVIEW

Proceedings of the Third Conference of the International Organization of Citrus Virologists. Edited by W. C. Price. University of Florida Press, Gainesville, Florida, 1965.

Price $ 8.00

This volume includes 69 papers presented at the 3rd Conference of the International Organization of Citrus Virologists, held at Campinas and Sao Paulo, Brazil in September 1963. Sixty-six papers have been published in this volume. Most of the authors who attended the Conference came almost from every citrus-growing part of the world. Like the first two volumes, the papers presented at this international gathering further evidenced a continued high degree of international co-operative efforts for solving baffling problems regarding citrus viruses throughout the world. These papers summarise to a large extent the progress that has been made in this field since the publication of the proceedings of the second conference held three years ago.

A large number of contributions are dealing with the four most important diseases of citrus, namely, tristeza, psorosis, xyloporosis and exocortis. Some papers are devoted to other viruses and virus-like symptoms as well as to the general survey of viroses in various citrus growing areas. Special papers have been contributed dealing with certain basic studies, the nucellar clones and root-stock and budwood certification; these contributions indicate a wide range of the diversified avenues of progress made within a short span of time. The present volume contains more papers dealing with the basic line of research. It opens with two papers on the purification and electron microscopy of "tristeza". The virus is now considered to have longest particles known so far. Besides, new methods have been also described in the field of mechanical transmission and short-time indexing. The papers contributed from Brazil indicate the advanced stage reached in the work of producing, selecting and distributing the nucellar clones of commercial varieties. The possibilities of cross-protection in the presence of milder strains has also been discussed. Special attention is given to certain pysiological aspects of virus diseases of citrus. Other problems concerned with stem-pitting in several varieties of citrus and seed transmission have been exhaustively dealt with.

The publication of such valuable information, consolidated in the form of a volume, would not only promote rapid dissemination of the recent advances in the field of citrology but also stimulate new ideas in the various techniques in research work. It would also serve the purpose of being a very useful reference-book for all the plant pathologists, especially those dealing with citrus virus diseases, as well as a handbook to the well informed nurserymen, growers and citriculturists.

S. P. RAYCHAUDHURI

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

Phytopathological News

CONGRATULATIONS to A. B. Joshi on his appointment as Deputy Director General, I. C. A. R., Krishi Bhavan, New Delhi.

CONGRATULATIONS to M. S. Swaminathan on his appointment as Director, I. A. R. I., New Delhi.

RETIREMENT : R. N. Tandon, Professor and Head of the Department of Botany, Allahabad University has retired after a long and meritorius service and continues to be associated with that institution as Professor Emeritus.

K. D. Bagchee after his retirement as Chief Mycologist, Forest Research Institute, Dehra Dun, continues to work in the same Institute as Emeritus Professor.

M. J. Thirumalachar, Chief Mycologist, Hindustan Antibiotics, Pimpri (Poona) proceeded on a visit to Mexico and U.S.A. for participating in the discussions on research and production of antibiotics and to visit Research Laboratories of Sherman and Upjohn Companies.

पुस्तकालय
078119
गुरुकुल कांगड़ी विश्वविद्यालय हरिद्वार

पुस्तकालय
गुरुकुल कांगड़ी, हरिद्वार

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

(Cont. from 2nd cover)

PAGES

Phytopathological Notes

A new blight disease of brinjal caused by Myrothecium roridum Tode ex Fr.—*Ram Nath, M. L. Seth and S. P. Raychaudhuri* ... 224

Aphelenchoides Ritzemabosi Parasitic on Zinnia elegans at Simla, India.—*S. T. Ahmad, Sheodhan Singh and D. P. Misra* ... 225

Association of the fungus Fusarium moniliforme Sheld. with the malformation in mango (Mangifera indica L.)—*A. S. Summanwar, S. P. Raychaudhuri and S. C. Phatak* ... 227

Lycogala mysorensis (Agnihothrudu) Comb. nov. (syn. Licea mysorensis Agnihothrudu.)—*V. Agnihothrudu* ... 229

Studies on the Host-Range of Uromyces Fabae (Pers.) De Bary—*R. G. Kapooria and S. Sinha* ... 229

Additions to the Fungi of Himachal Pradesh—*H. S. Sohi and G. K. Gupta* ... 230

Collar and root rot of 'ajowan'—*A. S. Prabhu* ... 231

Influence of cultural practices on the population of Meloidogyne javanica (Treub, 1995) Chitwood, 1949.—*R. B. S. Birat* ... 233

Powdery Mildews from Himachal Pradesh-I.—*H. S. Sohi, S. L. Sharma and K. B. Sachdeva* ... 235

Influence of soil temperature on infection of sugarcane by the smut fungus Ustilago scitaminea Syd.—*A. K. Lambat, V. V. Chenulu and B. L. Chona* ... 237

A Preliminary note on the Plant Parasitic Nematodes of the Mysore State. *B. C. N. Swamy and H. C. Govindu* ... 239

Antifungal activity of streptomycin.—*M. R. S. Iyengar* ... 241

Book Review—*S. P. Raychaudhuri* ... 243

Phytopathological News ... 244

RATES FOR REPRINTS

No. of copies—	25	50	75	100	150	200	250	300	400	500
2 Pages	2.50	3.00	3.50	4.00	5.60	7.00	8.50	10.00	12.50	15.00
4 ,,	5.00	6.00	7.00	8.00	10.00	12.00	15.00	18.00	24.00	30.00
8 ,,	10.00	12.00	14.00	16.00	20.00	24.00	30.00	38.00	50.00	60.00
Cover	3.00	4.50	5.00	6.00	9.00	11.00	14.00	16.00	20.00	25.00
Cost of Foreign Art Paper with Printing										
2 Pages	3.12	4.25	5.35	6.50	9.75	13.00	16.00	19.00	26.00	32.00

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

COUNCIL FOR 1966

President	... ...	R. N. TANDON
Immediate Past President		M. J. THIRUMALACHAR
Vice-President	... ...	K. S. BHARGAVA
Secretary-Treasurer	... ...	S. P. RAYCHAUDHURI (1965-67)
Joint Secretary	... ..	M. M. PAYAK (1965-67)

COUNCILLORS

Northern Zone	... ...	P. R. MEHTA
Mid-Eastern Zone	... ...	B. S. BAJAJ
Eastern Zone	... ...	B. GANGULY
Central Zone	... ...	T. SREERAMULU
Western Zone	... ...	M. C. SRINIVASAN
Southern Zone	... ...	K. RAMAKRISHNAN

EDITORIAL BOARD FOR 1965—67

R. PRASADA

Editor-in-Chief

T. S. SADASIVAN
V. P. BHIDE
KISHEN SINGH
S. P. RAYCHAUDHURI
B. L. RENFRO
V. V. CHENULU

L. M. JOSHI

INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing, but for others the annual subscription is Rs. 40/- (£ 3-10-0 or $ 9.00), post free, and payable in advance. Subscriptions should be sent with the order to the Secretary-Treasurer, THE INDIAN PHYTOPATHOLOGICAL SOCIETY, Indian Agricultural Research Institute, New Delhi-12 (India).

Edited & Published by Dr. R. Prasada for the Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi-12 and Printed at KAPOOR ART PRESS, New Delhi-5

पुस्तकालय

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

सन्दर्भ पुस्तक
REFERENCE BOOK

यह पुस्तक वितरित न की जाय
NOT TO BE ISSUED

Compiled
1999-2000

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri

CC-0. In Public Domain. Gurukul Kangri Collection, Haridwar

Digitized by Arya Samaj Foundation Chennai and eGangotri